Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

OPEN ACCESS

Assessment of antioxidant activity of ethanol extracts of *Vigna* spp.

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Article Details:
Received:2022-07-01
Accepted:2022-07-28
Available online:2022-11-30

DOI: https://doi.org/10.15414/ainhlq.2022.0013

Plants of *Vigna* Savi genus are widely used in the world as food and medicinal plants. Most investigations propagated the biochemical composition and biological activity of seeds of these plants due to the high content of proteins but fewer studies about the above-ground plant part. The aim of this study focused on the investigation of the antioxidant activity of *Vigna* spp. The plant raw of *Vigna angularis* (Willd.) Ohwi & H. Ohashi, *V. mungo* (L.) Hepper, *V. radiata* (L.) Wilczek, *V. unguiculata* (L.) Walp. was collected from the M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine experimental collection at the start of the vegetation and flowering stage. At the start of vegetation was determined 45.27–77.21 mg GAE.g^{-1} (gallic acid equivalent) of total polyphenol compounds (TPC), 8.67–20.48 mg CAE.g^{-1} (caffeic acid equivalent) of total phenolic acids (TPAC), 31.84–47.97 mg QE.g^{-1} (quercetin equivalent) of total flavonoids content (TFC), 6.97–8.14 mg TE.g^{-1} (Trolox equivalent) of antioxidant activity by DPPH method, and 110.52 to 142.61 mg TE.g^{-1} of AA by phosphomolybdenum method. At the flowering stage found the following results: 27.16–78.11 mg GAE.g^{-1} of polyphenol compounds, 4.97–17.16 mg CAE.g^{-1} of phenolic acids, 18.27–54.26 mg QE.g^{-1} of flavonoids, 4.6–6.69 mg TE.g^{-1} of AA by DPPH method, and 45.16–110.27 mg TE.g^{-1} of antioxidant activity by phosphomolybdenum method. A very strong correlation found between antioxidant activity by phosphomolybdenum method and TPAC (r = 0.883), TPC (r = 0.858), and TFC (r = 0.843) at the flowering stage. These results can be used for further biochemical and pharmacological investigations of these plants.

Keywords: *Vigna*, polyphenols, phenolic acids, flavonoids, correlation

Introduction

Representatives of *Vigna* L. genus relate to Fabaceae Lindle. plant family and consisting of more than 200 species (Harouna et al., 2020). These plants are widely distributed in tropical and subtropical regions and represented more than 80 species (Popoola et al., 2015). *Vigna* spp. is one of the most economically important plants in the world due to the wide use of seeds that is a rich source of protein (Musah et al., 2020). According to Dakora and Belane (2019), the content of leaf protein was 23–40% and seed protein up to 40%. Also, leaves and seeds are a rich source of macro- and microelements. The productivity of seeds in the conditions of the Right-Bank Forest Steppe of

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Ukraine is 486–586 g per square meter (Bondarchuk et al., 2022).

Plant raw material of *Vigna* is a rich source of protein (Sha'a, 2021), vitamins, sugars, and lipids (Vergun et al., 2022). According to Zi-Ul-Haq et al. (2014), seeds of *V. mungo* contain crude protein (25.07–28.60%), total lipids (5.13–6.22%), carbohydrates (54.81–58.13%), crude fibre (4.25–6.84%), ash (4.97–6.72%), amino acids, among which prevailed glutamic acid, aspartic acid, leucine, arginine, etc. Leaves of *V. unguiculata* contain saponins (1.34%), tannins (2.60%), terpenoids (0.47%), flavonoids (4.11%), alkaloids (3.55%), moisture (1.38%), ash (3.72%), etc. (Sha'a, 2021). As reported Wang et al. (2021), five principal fatty acids were found in seed raw such as palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid.

In addition, seed extracts of these plants exhibit various biological activities among which are antioxidant, antimicrobial, antidiabetic, hypocholesterolemic, antiviral, antifungal, thrombotic, etc. (Ibrahim et al., 2017). According to Lenny and Rizky (2020), leaves of *V. unguiculata* demonstrated antioxidant activity and were effective against *S. aureus* and *E. coli*.

Seeds of *Vigna* spp. widely used in Asian countries in food due to its rich nutritional composition and antioxidant effect (Siddhuraju and Becker, 2007; Wang et al., 2021) but exist very less information about the biochemical composition and biological activities of the above-ground part of these plants.

Taking this into account, the main goal of this study was to evaluate the antioxidant activity of the extracts of herbs of *Vigna* spp. as a potential source of polyphenol compounds.

Material and methodology

Biological material

The above-ground part of four species of the *Vigna* L. genus was used in this study. Plant raw of *Vigna angularis* (Willd.) Ohwi & H. Ohashi, *V. mungo* (L.) Hepper, *V. radiata* (L.) Wilczek, *V. unguiculata* (L.) Walp. were collected from an experimental collection of the M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine (Kyiv) at the start of the vegetation and flowering stage (Figure 1) in 2020–2021.

All biochemical analyses were conducted at the Slovak University of Agriculture in Nitra (Slovak Republic).

Chemicals

All chemicals used were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA) and CentralChem (Slovakia).

Preparations of extracts

An amount of 0.25 g of each sample was extracted with 20 mL of 80% ethanol for 2 h in a laboratory shaker GFL 3005 (GFL, Burgwedel, Germany). Then, the samples were centrifuged at 4605 RCF (Rotofix 32 A, Hettich, Germany) for 10 min and the supernatant was used for measurement of FRSA (antiradical activity) using DPPH, MRAP (antioxidant activity) using phosphomolybdenum method and measurement of other antioxidant properties (detection of total polyphenol, total flavonoid, and phenolic acid content).

Total polyphenol content of extracts

The total polyphenol content (TPC) was measured by the method of Singleton and Rossi (1965) using the

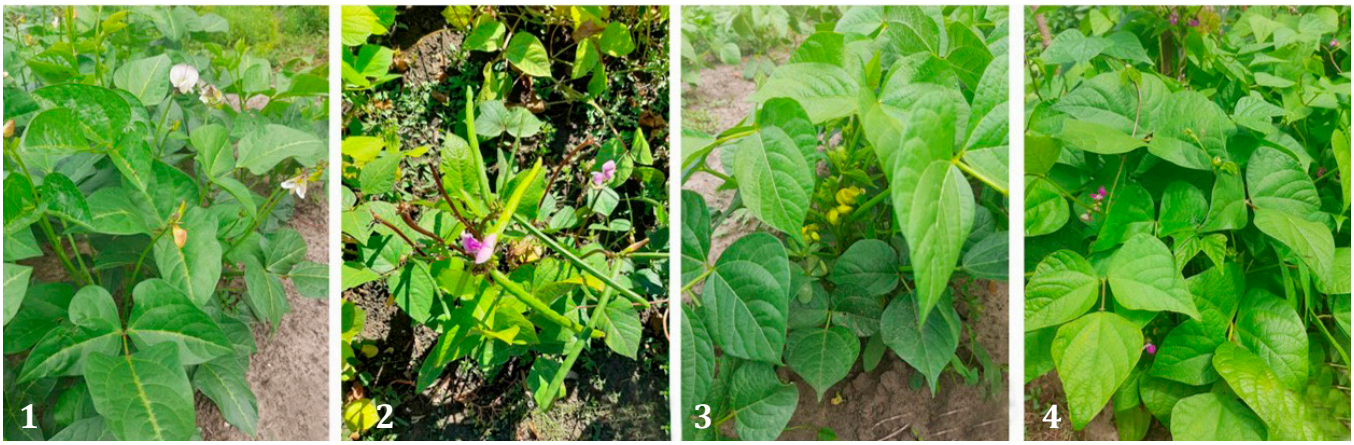

Figure 1 Plants of *Vigna* spp. at the flowering stage
1 – *Vigna angularis* (Willd.) Ohwi & H. Ohashi; 2 – *V. mungo* (L.) Hepper; 3 – *V. radiata* (L.) Wilczek; 4 – *V. unguiculata* (L.) Walp.

Folin-Ciocalteu reagent. A quantity of 0.1 mL of each sample was mixed with 0.1 mL of the Folin-Ciocalteu reagent, 1 mL of 20% (w/v) sodium carbonate, and 8.8 mL of distilled water. After 30 min in darkness, the absorbance at 700 nm was measured with the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25–300 $mg.L^{-1}$; R^2 = 0.998) was used as the standard. The results were expressed in $mg.g^{-1}$ DW gallic acid equivalent.

Total phenolic acid content

The content of phenolic acids was determined using Farmakopea Polska (1999). 0.5 ml of sample extract was mixed with 0.5 ml of 0.5 M hydrochloric acid, 0.5 ml Arnova reagent, 0.5 ml of 1 M sodium hydroxide (w/v), and 0.5 ml of distilled water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid 1–200 $mg.l^{-1}$ (R^2 = 0.999) was used as a standard. The results were expressed in $mg.g^{-1}$ caffeic acid equivalents (CAE).

Total flavonoid content of extracts

The total flavonoid content (TFC) was determined by the modified method described by Shafii et al. (2017). An aliquot of 0.5 mL of the sample was mixed with 0.1 mL of 10% (w/v) ethanolic solution of aluminium chloride, 0.1 mL of 1 M potassium acetate, and 4.3 mL of distilled water. After 30 min in darkness, the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (1–400 $mg.L^{-1}$; R^2 = 0.9977) was used as the standard. The results were expressed in $mg.g^{-1}$ DW quercetin equivalent.

Free radical scavenging activity

Free radical scavenging activity (FRSA) of samples (antiradical activity) was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sánchéz-Moreno et al., 1998). An amount of 0.4 mL of sample was mixed with 3.6 mL of DPPH solution (0.025 g DPPH in 100 mL ethanol). The absorbance of the reaction mixture was determined with the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10–100 $mg.L^{-1}$; R^2 = 0.989) was used as the standard and the results were expressed in $mg.g^{-1}$ DM Trolox equivalents.

Molybdenum reducing power of extracts

Molybdenum reducing power (MRP) of samples was determined by the method of Prieto et al. (1999) with slight modifications. The mixture of the sample (1 mL), monopotassium phosphate (2.8 mL, 0.1 M), sulfuric acid (6 mL, 1 M), ammonium heptamolybdate (0.4 mL, 0.1 M), and distilled water (0.8 mL) was incubated at 90 °C for 120 min, then cooled to room temperature. The absorbance at 700 nm was detected with the spectrophotometer Jenway (6405 UV/Vis, England). Trolox (10–1000 $mg.L^{-1}$; R^2 = 0.998) was used as the standard and the results were expressed in $mg.g^{-1}$ DM Trolox equivalent.

Statistical analysis

The results are expressed as mean values of three replications ± standard deviation (SD); hierarchical cluster analyses of similarity between samples were computed based on the Euclidean similarity index. Data were analyzed with the ANOVA test and differences between means were compared through the Tukey-Kramer test ($p < 0.05$).

Results and discussions

Plants belonging to the Fabaceae family include a large group of plants with antioxidant and antimicrobial properties (Obistioiu et al., 2021). The plant raw materials of Fabaceae plants are a rich source of polyphenol compounds (Doblado et al., 2005). Seeds of *Vigna* spp. plants along with other raw characterized by antioxidant activity due to the content of polyphenol compounds (Zi-Ul-Haq et al., 2013; Dalaram, 2015; Mahmoudi et al., 2020), and antioxidant properties depend on the processing of seeds (Yadav et al., 2018). According to Tungmunnithum et al. (2021), the total content of polyphenols in the seeds of *V. angularis* was 25.47–69.77 mg $GAE.g^{-1}$, *V. mungo* 26.35–54.72 mg $GAE.g^{-1}$, *V. radiata* 10.76–16.04 mg $GAE.g^{-1}$, and *V. unguiculata* 33.76–71.73 mg $GAE.g^{-1}$.

Polyphenols are bioactive and multi-functional compounds with antioxidant, anti-inflammatory, antitumoral, etc., activities (Cutrum and Cortez Sloboda, 2018). This group of compounds is the most abundant among antioxidants and plays an important role in human nutrition (Scalbert et al., 2005) and health benefits (Ignat et al., 2011). There are plant metabolites that are widely distributed in plants and plant products (Petti and Scully, 2009).

Previous studies of different raw Fabaceae representatives showed the high antioxidant potential of methanol, ethanol, and water extracts (Vergun et al., 2020a).

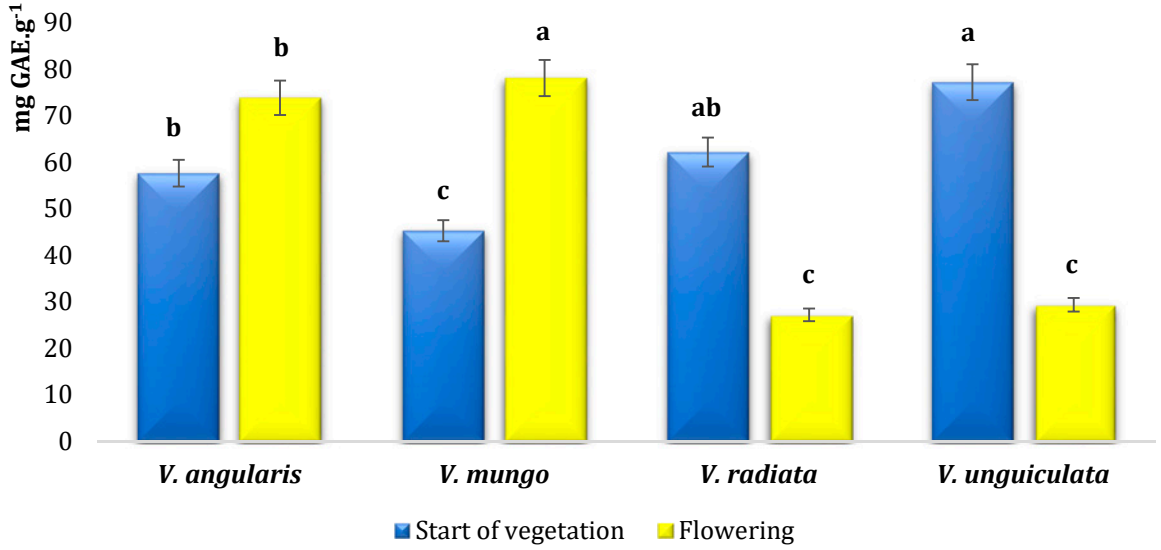

Figure 2 The content of polyphenol compounds in ethanol extracts of *Vigna* spp.
GAE – gallic acid equivalent; different superscripts in each column indicate the significant differences in the mean at p <0.05

The total content of polyphenol compounds at the start of vegetation of investigated plants was from 45.27 to 77.21 mg GAE.g^{-1} depending on the species (Figure 2). During the flowering period, polyphenol content in ethanol extracts of investigated species was from 27.16 to 78.11 mg GAE.g^{-1}.

According to Gođevac et al. (2008), the content of polyphenols in raw of nine Fabaceae species from natural flora was from 38 (*Coronilla emerus* L.) to 180.88 (*Lathyrus binatus* Pancic) mg GAE.g^{-1} depending on species. Total phenolic content of *Melilotus officinalis* (L.) Pall. was 21.37 mg GAE.g^{-1} in ethanol extracts (Mladenović et al., 2016). As reported Lee et al. (2018), polyphenol content in leaf extracts of *V. angularis* was in the range of 2.9–14.7 mg GAE.g^{-1}. The study of leaf extracts of other species *Desmodium canadensis* DC. showed that the total polyphenol content was 71.43 mg GAE.g^{-1} (Vergun et al., 2019).

Along with the polyphenol compounds study we used it to determine total phenolic acid content. Phenolic acids are a group of phenolic compounds that play an important role as antiaging agents and demonstrate antitumor, antimicrobial, and anti-inflammatory properties. These biologically active molecules are found in edible and nonedible plants (Jitan et al., 2018; Kumar and Goel, 2019). Phenolic acids released from emerging roots in Fabaceae plants during seed germination and in root nodules of *V. mungo* stimulate

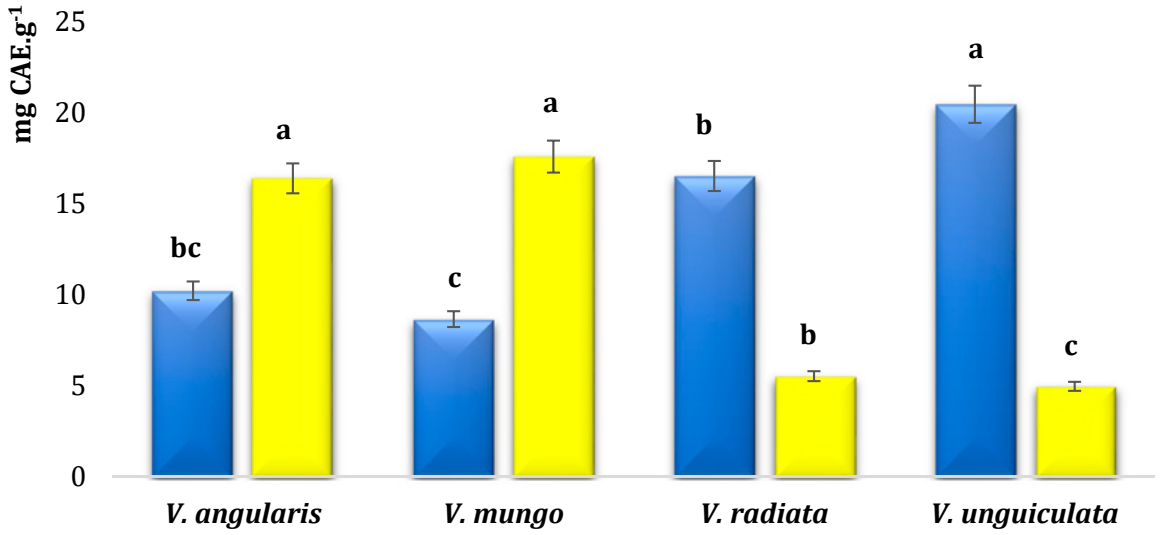

Figure 3 The content of phenolic acids in ethanol extracts of *Vigna* spp.
CAE – caffeic acid equivalent; different superscripts in each column indicate the significant differences in the mean at p <0.05

IAA production and nodules morphogenesis (Mandal et al., 2010).

The total content of phenolic acids in the ethanol extracts of investigated species was from 8.67 to 20.48 mg CAE.g^{-1} at the start of vegetation (Figure 3). This parameter was from 4.97 to 17.16 mg CAE.g^{-1} at the flowering stage depending on species. It should be noted that plants of *V. angularis* and *V. mungo* accumulated phenolic acids from the start of vegetation to the flowering stage and plants of *V. radiata* and *V. unguiculata* opposite.

The total phenolic acid content of ethanol extracts of *Galega* spp. was 14.13–16.73 mg CAE.g^{-1} at the start of vegetation and 11.62–16.22 mg CAE.g^{-1} at the flowering stage (leaves) (Vergun et al., 2020b). The study of leaf extracts of other species *Desmodium canadensis* showed that the total phenolic acid content was 8.70 mg CAE.g^{-1} (Vergun et al., 2019).

Also, we were used to detect the total flavonoid content in ethanol extracts of *Vigna* species. Flavonoids are a versatile class of natural compounds that demonstrated different biological activities such as antimicrobial and antifungal (Saleem et al., 2017). These polyphenol compounds are abundant in fruits, vegetables, and grains, have antioxidant, and anti-inflammatory activity and reduce the risk of diseases (Shen et al., 2022).

The total content of flavonoids in the ethanol extracts of investigated species of *Vigna* was from 31.84 to 47.97 mg QE.g^{-1} at the start of vegetation (Figure 4). The content of flavonoids was from 18.27 to 54.26 mg QE.g^{-1} during the flowering period depending on the species.

According to Berber et al. (2014), extracts of plants *Adenocarpus complicatus* (L.) Gay demonstrated 8.89 mg RE.g^{-1} (rutin equivalent) in fruits and 36.67 mg RE.g^{-1} of total flavonoid content in mixed raw. The ethanol extracts of other species from Fabaceae such as *Galega* spp. had a flavonoid content of 38.79–44.27 mg QE.g^{-1} at the start of vegetation and 40.09–44.91 mg QE.g^{-1} at the flowering stage (leaves) (Vergun et al., 2020b). The study of leaf extracts of other species *Desmodium canadensis* showed that the total flavonoid content was 61.05 mg QE.g^{-1} (Vergun et al., 2019). The content of flavonoids in seeds of *V. unguiculata* was from 30.5 to 46.3 mg RE.g^{-1} (rutin equivalent) (Nassourou et al., 2016).

The polyphenol compounds act as antioxidant agents and the antioxidant activity of plant raw is caused by the presence of these compounds. It exists numerous methods to determine it such as 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity (DPPH), ferric reducing assay (FRAP), Trolox equivalent antioxidant capacity, etc. (Moharram and Youssef, 2014; Chaves et al., 2020). The extracts of different plant parts such as leaves, stems, inflorescences, and fruits exhibited antioxidant potential depending on the species (Krishnaiah et al., 2011).

This study used two methods to evaluate the antioxidant activity such as DPPH and the phosphomolybdenum method which are, according to Alam et al. (2013), related to *in vitro* methods and also are the most widely used. A previous study about antioxidant activity by the DPPH method of Fabaceae species showed high values in the methanol and water extracts (Vergun et al., 2020a).

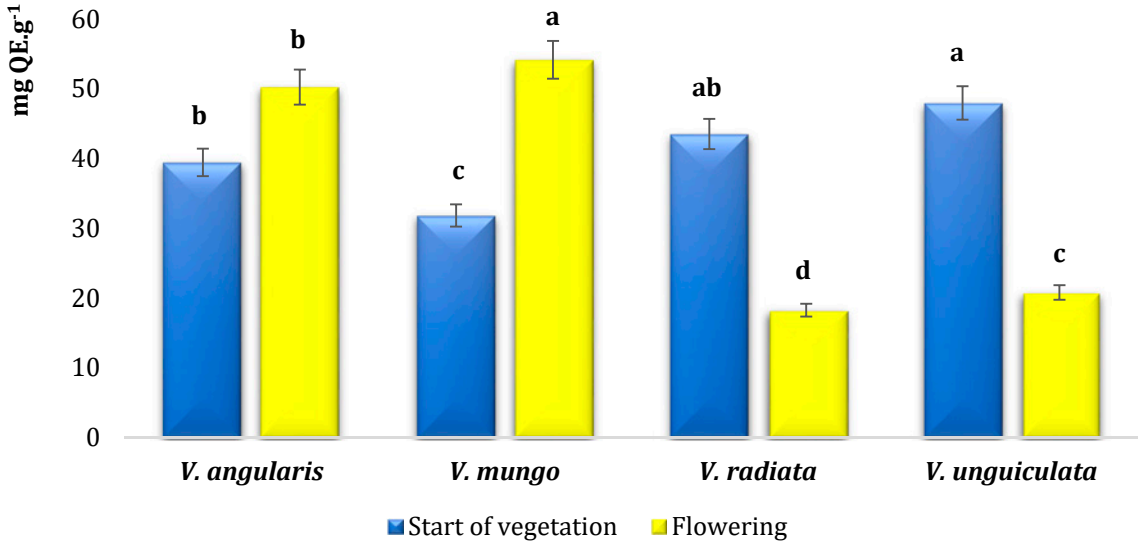

Figure 4 The content of flavonoids in ethanol extracts of *Vigna* spp.
QE – quercetin equivalent; different superscripts in each column indicate the significant differences in the mean at p <0.05

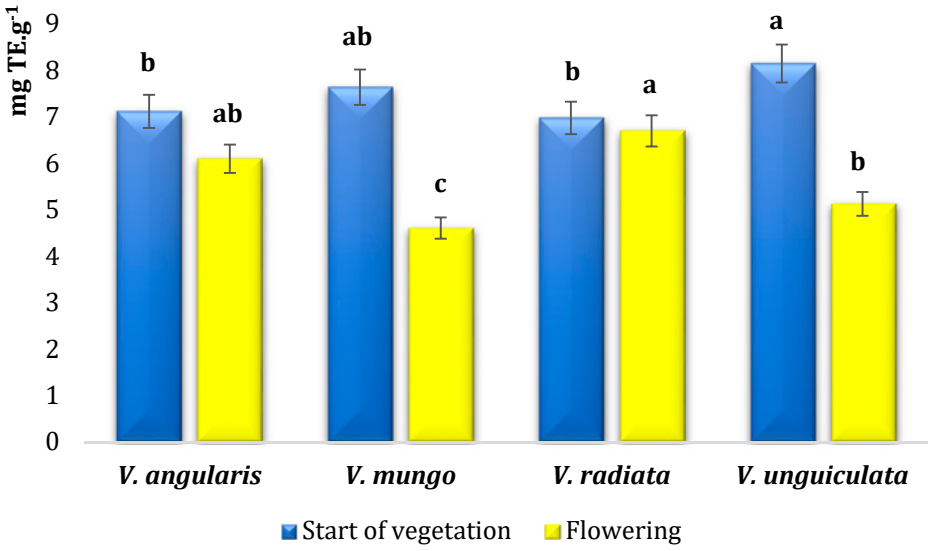

Figure 5 Antioxidant activity of ethanol extracts of *Vigna* spp. by DPPH method
TE – Trolox equivalent; different superscripts in each column indicate the significant differences in the mean at p <0.05

The antioxidant activity of investigated plant extracts by the DPPH method was from 6.97 to 8.14 mg TE.g^{-1} at the start of vegetation and from 4.6 to 6.69 mg TE.g^{-1} at the flowering stage (Figure 5).

The phosphopmolybdenum method of antioxidant activity determination is based on a redox antioxidant reaction where phosphate-Mo (VI) is reduced to phosphate-Mo (V) (Phatak and Hendre, 2014). As described Diwan et al. (2012), the DPPH scavenging assay usually detected the polyphenols and flavonoids, and the phosphomolybdenum assay is used for some phenolics, usually its ascorbic acid, carotenoids, etc. The antioxidant activity of investigated plant extracts by the phosphomolybdenum method was from 110.52 to 142.61 mg TE.g^{-1} at the start of vegetation and from 45.16 to 110.27 mg TE.g^{-1} at the flowering stage (Figure 6).

As reported Berber et al. (2014), *Adenocarpus complicatus* extracts had antioxidant activity by phosphomolybdenum method 207.53 mg TE.g^{-1} in fruits and 251.53 mg TE.g^{-1} in the mixed raw.

The study of leaf extracts of other species *Desmodium canadensis* showed that antioxidant activity by the phosphomolybdenum method was 190.64 mg TE.g^{-1} (Vergun et al., 2019).

The correlation analyses between antioxidant parameters were conducted (Table 1). A very strong

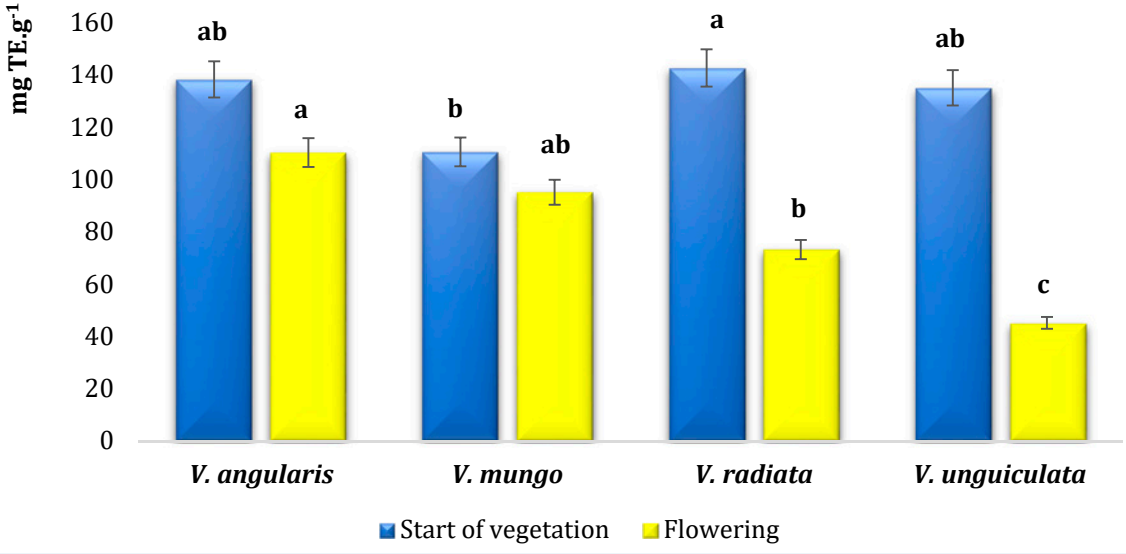

Figure 6 Antioxidant activity of ethanol extracts of *Vigna* spp. by phosphomolybdenum method
TE – Trolox equivalent; different superscripts in each column indicate the significant differences in the mean at p <0.05

Table 1 Correlation analysis between antioxidant parameters of *Vigna* spp.

Parameter	TPC	TPAC	TFC	DPPH	PHOMO
Start of vegetation					
TPAC	**0.937****	**1**	**0.938****	**0.405***	**0.577***
TFC	**0.976****	**0.938****	**1**	**0.227***	**0.793****
DPPH	**0.423***	**0.405***	**0.227***	**1**	**-0.389**
PHOMO	**0.663****	**0.577***	**0.793****	**-0.389**	**1**
Flowering					
TPAC	**0.997****	**1**	**0.996****	**-0.36***	**0.883****
TFC	**0.999****	**0.996****	**1**	**-0.433**	**0.843****
DPPH	**-0.405**	**-0.365**	**-0.433**	**1**	**0.111***
PHOMO	**0.858****	**0.883****	**0.843****	**0.111***	**1**

Note: TPC – total phenolic content; TPAC – total phenolic acid content; TFC – total flavonoid content; DPPH – antioxidant activity by DPPH method; PHOMO – antioxidant activity by phosphomolybdenum method. ** Correlation is significant at p ≤0.01; * correlation is significant at p ≤0.05

correlation at the start of vegetation found between TPC and TFC (r = 0.976), TPAC and TFC (r = 0.938) and TPC and TPAC (r = 0.937). A strong correlation at the start of vegetation was detected between TFC and PHOMO (r = 0.793) and TPC and PHOMO (r = 0.663). Between the content of all phenolic compounds and antioxidant activity by the DPPH method at the start of vegetation determined a moderate or weak correlation (r = 0.227–0.423). The negative correlation between antioxidant activity by DPPH and phosphomolybdenum method (r = -0.389).

A very strong correlation was found between the following investigated parameters at the flowering stage: TPC and TFC (r = 0.999), TPC and TPAC (r = 0.997), TPAC and TFC (r = 0.996). A very strong correlation was found between PHOMO and all phenolic compound groups investigated in this study (r = 0.843–0.883). Negative relations were found between antioxidant activity by the DPPH method and investigated parameters.

Due to existing fewer data about correlation analysis between antioxidant parameters of *Vigna* spp. above-ground part, it is difficult to compare obtained results. A negative correlation between antioxidant activity by two methods such as DPPH and phosphomolybdenum was found in another study (Kasangana et al., 2015). According to Lee et al. (2018), the leaves extracts study of *V. angularis* demonstrated a negative correlation between DPPH scavenging activity and total phenolic content (r = -0.722) whereas in our study this correlation was moderate.

Conclusions

Taking the obtained data into account it should be noted that investigated species of the *Vigna* genus are a good source of antioxidants. The study of ethanol extracts of above-ground parts of four species showed some patterns in the accumulation of selected polyphenol compounds. So, the accumulation of total polyphenol compounds, phenolic acids, and flavonoids in *V. angularis* and *V. mungo* extracts was higher at the flowering stage than at the start. The opposite was indicated for extracts of *V. radiata* and *V. unguiculata*, where all investigated polyphenol compounds were higher at the start of vegetation. The antioxidant activity by the phosphomolybdenum method was less at the flowering stage for all investigated species. In this study, a very strong correlation was found between polyphenol compounds and antioxidant activity by the phosphomolybdenum method at the flowering stage, whereas relations between polyphenols and the DPPH method of antioxidant activity determination were weaker. These results can be used for further biochemical and pharmacological investigations.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

Acknowledgements

The publication was prepared with the active participation of researchers in International Network AgroBio*Net*.

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

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Biomarkers of oxidative stress in the muscle tissue of atlantic salmon (*Salmo salar* L.) treated *in vitro* by extracts of *Chelidonium majus* L.

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Article Details:
Received:2022-07-04
Accepted:2022-08-01
Available online:2022-11-30

DOI: https://doi.org/10.15414/ainhlq.2022.0014

Consistent with our previous studies, we continue to evaluate the antioxidant potential of representatives belonging to the Papaveraceae family collected from the northern part of Poland using a muscle tissue model of Atlantic salmon (*Salmo salar* L.). Therefore, in the present study, oxidative stress biomarkers (2-thiobarbituric acid reactive substances (TBARS), aldehydic and ketonic derivatives of oxidatively modified proteins (OMP), total antioxidant capacity (TAC)) and also activity of antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase) were used for evaluating the *in vitro* antioxidant activity of root and stalk extracts derived from great celandine *Chelidonium majus* L. (CM) collected in urban and rural agglomerations of Kartuzy district (Pomeranian province, northern part of Poland). Freshly collected roots and stalks were washed, weighed, crushed, and homogenized in 0.1 M phosphate buffer (pH 7.4) (in the proportion of 1:19, w/w) at room temperature. Incubation of salmon muscle tissue with extracts derived from both the stalks and roots of CM harvested from rural areas resulted in a decrease in lipid peroxidation. The incubation of salmon muscle tissue with extracts derived from both the stems and roots of CM harvested from rural areas resulted in a decrease in lipid peroxidation. Similarly, the use of extracts derived from the roots of CM collected from urban areas resulted in a decrease in TBARS levels. These results suggest that it can be argued that the presence of secondary plant metabolites in CM extracts protects structures of cell membranes against the damaging effects of free radicals. On the other hand, analysis of levels of protein oxidation after incubation of muscle tissue with CM extracts showed that extracts derived from both roots and stalks of CM harvested from urban areas reduced levels of ketonic derivatives of oxidatively modified proteins. Analyzing the total antioxidant capacity after the incubation with CM extracts under *in vitro* conditions, we concluded that extracts mainly derived from the stalks of CM harvested from both urban and rural areas effectively increase TAC levels. These results are reflected after analysis of antioxidant enzyme activity, where we observed statistically significant increases in superoxide dismutase and catalase activity. On the other hand, the incubation of CM extracts with muscle tissue resulted in a statistically significant decrease in glutathione peroxidase activity compared to the control samples.

Keywords: root and stalk extracts, Atlantic salmon, muscle tissue, biomarkers of oxidative stress, antioxidant enzymes

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Introduction

Many proteins contain redox-sensitive thiols, and reactions of thiol systems occur largely by non-radical two-electron transfers. Accumulating data show that central thiol-disulfide couples are maintained under non-equilibrium conditions in biological systems. This presents a condition wherein changes in abundance and distribution of redox catalysts and changes in rates of generation of relevant oxidants (e.g., peroxides) and precursors for NADPH supply can account for pathological effects of oxidative stress through altered functions of enzymes, receptors, transporters, transcription factors, and structural elements, without free radicals (Go and Jones, 2013). Free radicals or reactive oxygen species (ROS) are generated by oxygen metabolism which is balanced by the rate of oxidant formation and the rate of oxidant elimination (Sinha and Dabla, 2015). Oxidative stress is a result of an imbalance between the generation of reactive oxygen species and the antioxidant defence systems. Oxidative stress is involved in the development and progression of clinical and experimental tissue failure (Sies, 2015; Herrmann and Dick, 2012). Oxidative stress is defined as a dysregulation between the production of reactive oxygen species and the endogenous antioxidant defence mechanisms, the so-called 'redox state'. When present in low concentrations, ROS plays a critical function in cell homeostasis. However, excess ROS causes cellular dysfunction, protein and lipid peroxidation, and DNA damage, and eventually leads to irreversible cell damage and death (Khatri et al., 2018). Oxidative stress is predominantly caused by a host of lifestyle-related factors, the majority of which are modifiable. Antioxidant regimens and lifestyle modifications could both be plausible therapeutic approaches that enable the burden of oxidative stress-induced tissue damage to be overcome (Jones, 2006; van der Pol et al., 2018).

Better knowledge of oxidative balance in fish tissues and its application to fisheries and aquaculture science (i.e., breeding fit fish) is needed in the face of global environmental change, high fishing pressure, increased aquaculture production, as well as increased concern for fish welfare (Johnston, 1999; Palstra and Planas, 2011). Oxidative stress-related diseases can contribute to increased fish mortality (Bisht et al., 2017). Therefore, there is a need to search for measurements to prevent oxidative imbalance in fish. It is suggested that medicinal plants containing secondary metabolites such as alkaloids, polyphenols, and vitamins, among others, can contribute to eliminating the harmfulness of oxidative stress (Koleva et al., 2018). Recent scientific reports have demonstrated that plants belonging to the Papaveraceae family contain several compounds possessing antioxidant properties. Great celandine *Chelidonium majus* L. (CM) (Papaveraceae) has a long history of being useful for the treatment of many diseases. This plant is of great interest for its use also in Chinese herbal medicine (Zielińska et al., 2018). The plant contains as major secondary metabolites isoquinoline alkaloids, such as sanguinarine, chelidonine, chelerythrine, berberine, and coptisine. Other compounds structurally unrelated to the alkaloids have been isolated from the aerial parts: several flavonoids and phenolic acids. CM extracts and their purified compounds exhibit antiviral, antitumor, antimicrobial, and antioxidative properties in both *in vitro* and *in vivo* studies (Arora and Sharma, 2013).

Therefore, the current study aimed to assess the *in vitro* antioxidant activity of root and stalk extracts derived from CM collected in urban and rural agglomerations of Kartuzy district (Pomeranian province, northern part of Poland). For this purpose, we used the oxidative stress biomarkers (2-thiobarbituric acid reactive substances, carbonyl derivatives of oxidative modification of proteins, total antioxidant capacity), as well as activity of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) in the muscle tissue of Atlantic salmon (*Salmo salar* L.) incubated *in vitro* with CM extracts.

Materials and methodology

Collection of plant material

Plant materials were harvested from natural habitats on the territory of the Kartuzy district (54° 20′ N 18° 12′ E) in the Pomeranian province (northern part of Poland). The plant collection covered the period from June to July 2020. For our studies, we collected CM plants in phases beginning with flowering (flower buds visible) and full flowering (yellow flowers blooming, young fruits small and developing). Kartuzy is located about 32 kilometres (20 miles) west of Gdańsk and 35 km (22 miles) south-east of Lębork town on a plateau at an altitude of approximately 200 meters (656 feet) above sea level on average. The plateau, which is divided by the Radaune lake, comprises the highest parts of the Baltic Sea Plate (http://www.kartuzy.pl/). Plants were collected from urban (n = 5) and rural agglomerations (n = 15) on the territory of the Kartuzy district.

Preparation of plant extracts

Freshly collected roots and stalks were washed, weighed, crushed, and homogenized in 0.1M phosphate

buffer (pH 7.4) (in the proportion of 1:19, w/w) at room temperature. The extracts were then filtered and used for analysis. The extracts were stored at -25 °C until use.

Experimental fish and muscle tissue samples

Clinically healthy Atlantic salmon (*Salmo salar* L.) with a mean body mass of 85–190 g were used in the experiments. The fish samples for the current study were carried out in the Department of Salmonid Research, Inland Fisheries Institute (Rutki, Poland). The muscle tissues were sampled after the decapitation of the fish. The minced muscle tissue was rinsed clear of blood with cold isolation buffer (100 mM Tris-HCl, pH 7.2) and homogenized in a homogenizer H500 with a motor-driven pestle on ice. Homogenates were centrifuged at 3,000 rpm for 15 min at 4 °C. After centrifugation, the supernatant was collected and frozen at -25 °C until analyzed. Protein contents were determined with the method described by Bradford (1976) with bovine serum albumin as a standard. Absorbance was recorded at 595 nm. All enzymatic assays were carried out at 22 ±0.5 °C using a Specol 11 spectrophotometer (Carl Zeiss Jena, Germany) (n = 8). The enzymatic reactions were started by adding the tissue supernatant.

Experimental design

The supernatant of the muscle tissue was used to incubate with extracts obtained from roots and stalks of CM (in a final concentration of extracts of 2.5 mg per mL) at room temperature. The control untreated samples (muscle tissue) were incubated only with 100 mM Tris-HCl buffer (pH 7.2) (in the same ratio). The incubation time was 2 hours. Biomarkers of oxidative stress and antioxidant defences were studied in the incubated homogenate (control untreated group and in samples with extracts obtained from roots and stalks of CM).

The 2-Thiobarbituric acid reactive substances (TBARS) assay

The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reacting substances (TBARS) with the Kamyshnikov (2004) method for determining the malonic dialdehyde (MDA) concentration. This method is based on the reaction of the degradation of the lipid peroxidation product, MDA, with 2-thiobarbituric acid (TBA) under high temperature and acidity to generate a coloured adduct that is measured spectrophotometrically. The nmol of MDA per mg of protein was calculated using $1.56 \cdot 10^5$ $mM^{-1}.cm^{-1}$ as the extinction coefficient.

The carbonyl derivatives content of protein oxidative modification (OMP) assay

To evaluate the protective effects of extracts derived from roots and stalks of CM collected in urban and rural agglomerations against free radical-induced protein damage in the muscle tissue of Atlantic salmon, a content of aldehydic and ketonic derivatives of oxidative modification of proteins (OMP) based on the spectrophotometric measurement was done. The rate of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives of amino acid reaction with 2,4-dinitrophenylhydrazine (DNFH) as described by Levine and co-workers (1990) and as modified by Dubinina and co-workers (1995). DNFH was used for determining carbonyls in soluble and insoluble proteins. Carbonyl groups were determined spectrophotometrically from the difference in absorbance at 370 nm (aldehydic derivatives, OMP_{370}) and 430 nm (ketonic derivatives, OMP_{430}).

Measurement of total antioxidant capacity (TAC)

The TAC level in samples was estimated by measuring the 2-thiobarbituric acid reactive substances (TBARS) level after Tween 80 oxidation. This level was determined spectrophotometrically at 532 nm (Galaktionova et al., 1998). The sample inhibits the Fe^{2+}/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. The level of TAC in the sample (%) was calculated concerning the absorbance of the blank samples.

Measurement of superoxide dismutase activity

Superoxide dismutase (SOD, E.C. 1.15.1.1) activity was assessed by its ability to dismutate superoxide produced during quercetin auto-oxidation in an alkaline medium (pH 10.0) by Kostiuk et al. (1990) method. Activity is expressed in units of SOD per mg of protein.

Measurement of catalase activity

Catalase (CAT, E.C. 1.11.1.6) activity was determined by measuring the decrease of H_2O_2 in the reaction mixture using a spectrophotometer at the wavelength of 410 nm by the method of Koroliuk et al. (1988). One unit of catalase activity is defined as the amount of enzyme required for the decomposition of 1 μmol H_2O_2 per min per mg of protein.

Measurement of glutathione peroxidase activity

Glutathione peroxidase (GPx, EC 1.11.1.9) activity was determined by detecting the non-enzymatic utilization of GSH (the reacting substrate) at an absorbance of 412 nm after incubation with 5,5-dithiobis-2-nitrobenzoic acid (DTNB) according to by the method of Moin (1986). The rate of GSH reduction was followed spectrophotometrically at 412 nm. GPx activity is expressed as nmol GSH per min per mg of protein.

Statistical analysis

The mean ± S.E.M. values were calculated for each group to determine the significance of the intergroup difference. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors test ($p > 0.05$). The significance of differences between the levels of oxidative stress biomarkers (significance level, $p < 0.05$) was examined using the Mann-Whitney *U* test (Zar, 1999). All statistical calculation was performed on separate data from each individual with Statistica 13.3 software (TIBCO Software Inc., Krakow, Poland).

Results and discussion

Figure 1 demonstrates the TBARS levels in the muscle tissue of Atlantic salmon after *in vitro* incubation with extracts derived from roots and stalks of CM collected from rural and urban areas of the Pomeranian region. Analyzing the TBARS levels after the *in vitro* treatment of CM extracts, we obtained the following results. There was a reduction in TBARS levels after *in vitro* incubation of salmon muscle tissue with root extracts of CM collected from rural areas (145.69 ±9.66 nmol.mg^{-1} protein) compared to the untreated control samples (154.56 ±7.1 nmol.mg^{-1} protein). There was a statistically no-significant decrease in TBARS levels by 5.82% ($p > 0.05$) compared to the controls (Figure 1).

We obtained similar results after *in vitro* incubation with root extracts of CM collected from urban agglomerations with salmon muscle tissue, where we also observed a statistically no-significant decrease in TBARS by 5.85% ($p > 0.05$) compared to the control samples (145.52 ±3.84 nmol.mg^{-1} protein vs. 154.56 ±7.1 nmol.mg^{-1} protein). The opposite trends were observed after *in vitro* incubation of salmon muscle tissue with stalk extracts of CM collected from urban areas. The use of stalk extracts of CM collected from urban areas (159.69 ±5.05 nmol.mg^{-1} protein) resulted in a statistically no-significant highest increase in TBARS levels (by 3.32%, $p > 0.05$) compared to the control samples (154.56 ±7.1 nmol.mg^{-1} protein). After *in vitro* incubation of salmon muscle tissue with stalk extracts of CM harvested from a rural agglomeration, we observed a statistically no-significant decrease in TBARS levels (by 3.03%, $p > 0.05$) compared to the control samples (149.88 ±3.58 nmol.mg^{-1} protein vs. 154.56 ±7.1 nmol.mg^{-1} protein) (Figure 1).

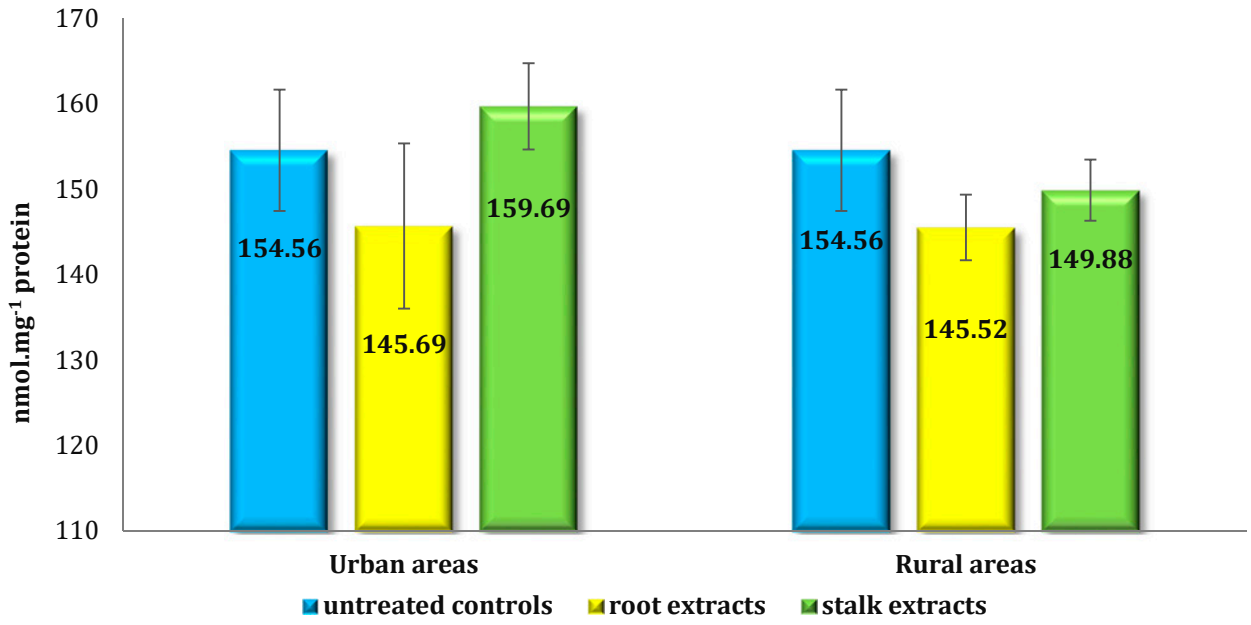

Figure 1 The TBARS level as a biomarker of lipid peroxidation in the muscle tissue of Atlantic salmon after *in vitro* incubation with root and stalk extracts of *Chelidonium majus* L. collected from rural and urban areas of the Pomeranian region (M ±m, n = 8)

The aldehydic and ketonic derivatives of oxidatively modified proteins in the muscle tissue of Atlantic salmon after *in vitro* incubation with extracts derived from roots and stalks of CM collected from rural and urban areas of the Pomeranian region were present in Figure 2.

Analyzing levels of protein oxidation after incubation of muscle tissue with CM extracts, we observed interesting results. We noted similar levels of aldehydic derivatives of oxidatively modified proteins after *in vitro* incubation of salmon muscle tissue with CM stalk extracts of CM collected from urban areas (14.24 ±0.12 nmol.mg^{-1} protein) compared with the control samples (14.7 ±0.27 nmol.mg^{-1} protein), where there was a statistically no-significant decrease by 3.13% (p >0.05). Similarly, *in vitro* incubation of muscle tissue with stalk extracts of CM harvested from rural

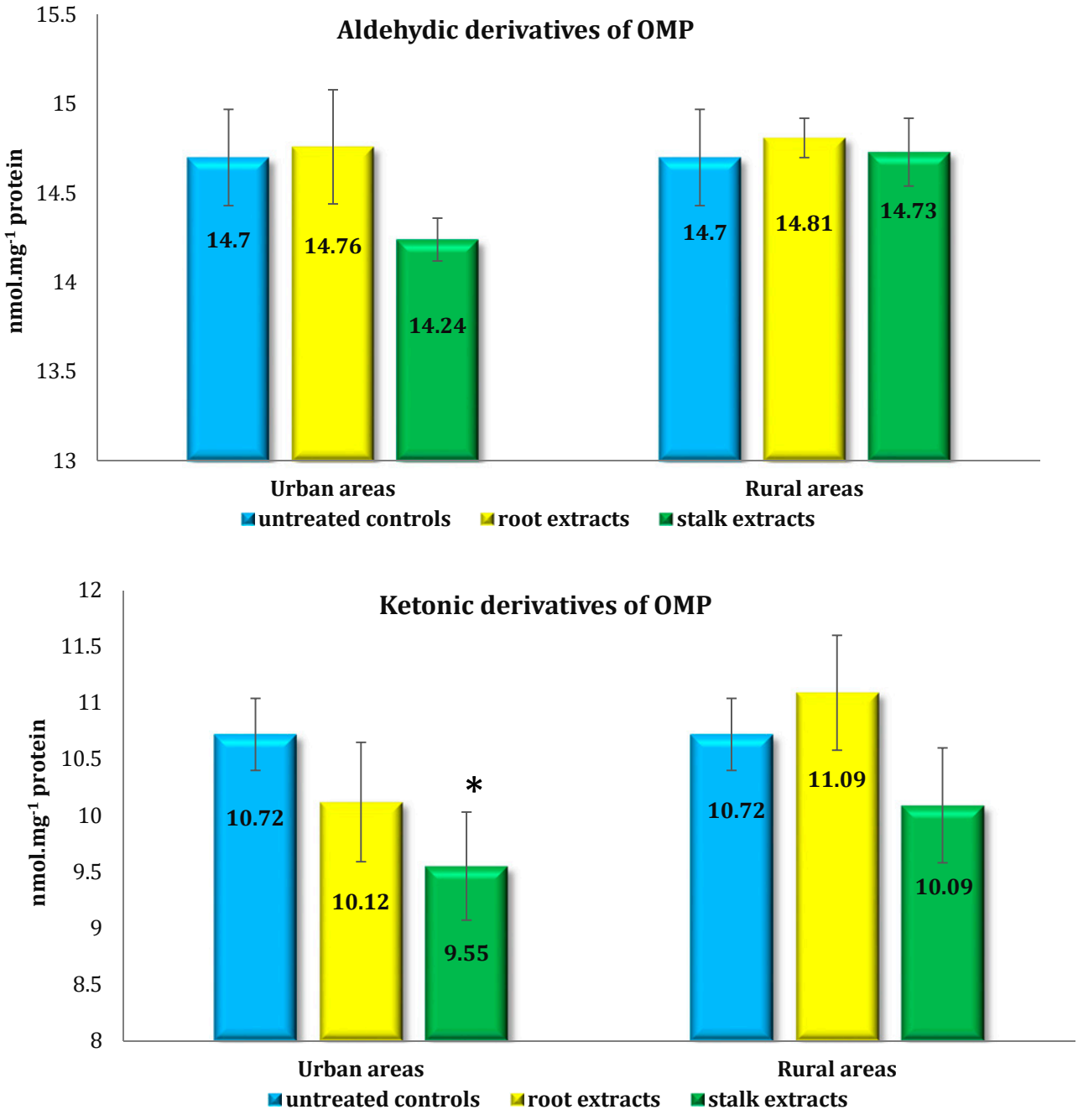

Figure 2 The aldehydic and ketonic derivatives of oxidatively modified proteins in the muscle tissue of Atlantic salmon after *in vitro* incubation with root and stalk extracts of *Chelidonium majus* L. collected from rural and urban areas of Pomeranian region (M ± m, n = 8)
*– statistically significant differences (p <0.05) compared to the control samples

areas resulted in similar levels of aldehydic derivatives of OMP compared to the controls (14.73 ±0.19 nmol. mg^{-1} protein vs. 14.7 ±0.27 nmol.mg^{-1} protein). After incubating the salmon muscle tissue with root extracts of CM collected from both urban and rural agglomerations, we also observed similar levels of aldehydic derivatives of OMP compared to the control samples (14.76 ±0.32 nmol.mg^{-1} protein vs. 14.7 ±0.27 nmol.mg^{-1} protein for extracts of CM collected from urban areas; 14.81 ±0.11 nmol.mg^{-1} protein vs. 14.7 ±0.27 nmol.mg^{-1} protein for extracts of CM collected from rural areas).

After *in vitro* incubation of salmon muscle tissue with stalk extracts of CM collected from urban areas, we recorded a statistically significant reduction in the level of ketonic derivatives of oxidatively modified proteins (by 10.91%, p <0.05) compared to the control samples (9.55 ±0.48 nmol.mg^{-1} protein vs. 10.72 ±0.32 nmol. mg^{-1} protein). We noted similar results after incubating salmon muscle tissue with root extracts of CM collected from urban areas, where there was also, but a statistically no-significant decrease (by 5.6%, p >0.05) in levels of ketonic derivatives of oxidatively modified proteins (10.12 ±0.53 nmol.mg^{-1} protein) compared to the control samples (10.72 ±0.32 nmol.mg^{-1} protein). Also, we recorded a statistically no-significant decrease in levels of ketonic derivatives of OMP after *in vitro* incubation of salmon muscle tissue with stalk extracts of CM collected from rural areas (by 5.88%, p >0.05) compared to the control samples (10.09 ±0.51 nmol.mg^{-1} protein vs. 10.72 ±0.32 nmol.mg^{-1} protein). Other results were obtained after *in vitro* incubation of muscle tissue with root extracts of CM collected from rural agglomerations, where we noted a statistically no-significant increase in levels of ketonic derivatives of OMP (by 3.45%, p >0.05) compared to the control samples (11.09 ±0.51 nmol.mg^{-1} protein vs. 10.72 ±0.32 nmol.mg^{-1} protein) (Figure 2).

The total antioxidant capacity in the muscle tissue of Atlantic salmon after *in vitro* incubation with extracts derived from roots and stalks of CM collected from rural and urban areas of the Pomeranian region is presented in Figure 3.

When measuring total antioxidant capacity, we observed a statistically significant increase in TAC levels (by 18.86%, p <0.05) after *in vitro* incubation of muscle tissue with stalk extracts of CM collected from urban areas (31.39 ±3.37%) compared to the control samples (26.41 ±1.83%). Similar results were also obtained after incubation of muscle tissue with stalk extracts of CM collected from rural agglomerations (31.59 ±1.24%), where there was a statistically significant increase in TAC levels by 19.61% (p <0.05) compared to the control samples (26.41 ±1.83%). Incubation of muscle tissue with root extracts of CM collected from both urban (30.66 ±1.72%) and rural

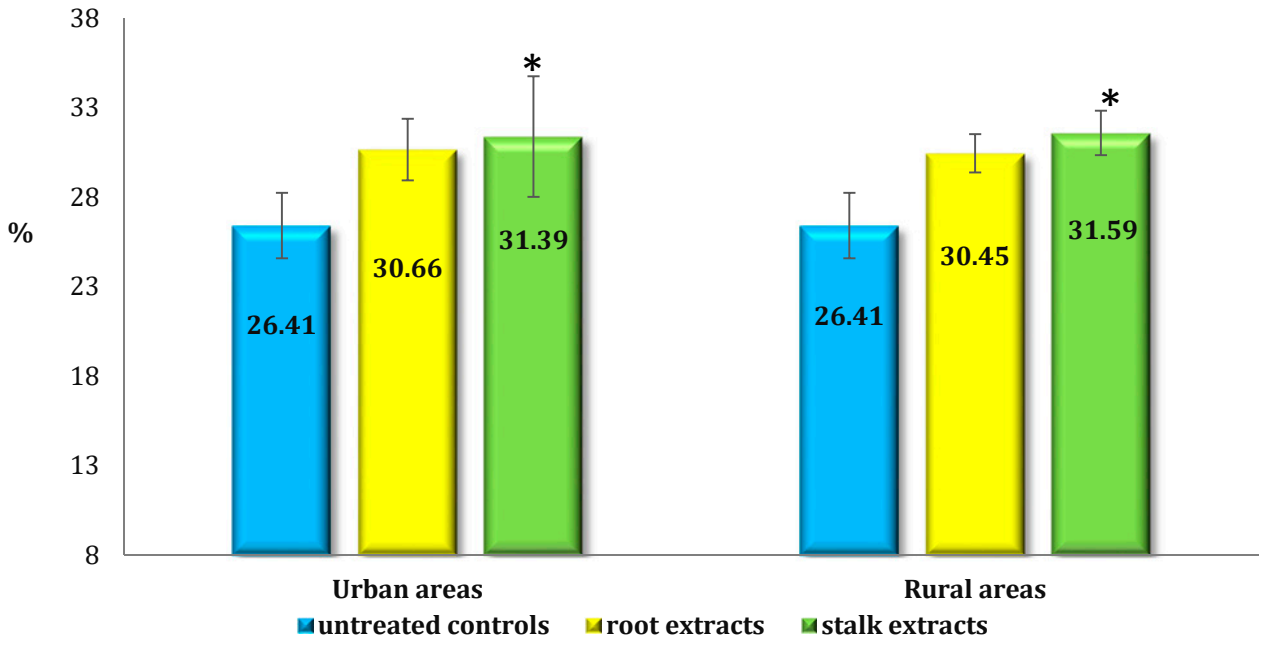

Figure 3 The total antioxidant capacity in the muscle tissue of Atlantic salmon after *in vitro* incubation with root and stalk extracts of *Chelidonium majus* L.CM collected from rural and urban areas of Pomeranian region (M ±m, n = 8)
*– statistically significant differences (p <0.05) compared to the control samples

areas (30.45 ±1.07%) also resulted in a statistically no-significant increase in total antioxidant capacity by 16.09% (p >0.05) for extracts of CM collected from urban areas and by 15.3% (p >0.05) for extracts of CM collected from rural areas compared to the control samples (26.41 ±1.83%) (Figure 3).

The superoxide dismutase activity in the muscle tissue of Atlantic salmon after *in vitro* incubation with extracts derived from roots and stalks of CM collected from rural and urban areas are presented in Figure 4.

When we examined the activity of superoxide dismutase in the muscle tissue of salmons incubated *in vitro* with stalk extracts of CM collected from rural agglomerations, we recorded the highest activity of this antioxidant enzyme with a value of (398.68 ±23.38 U.mg^{-1} protein) compared to the control samples (353.5 ±16.69 U.mg^{-1} protein). There was a statistically no-significant increase in SOD activity by 12.8% (p >0.05) compared to the control samples. Similar results were obtained after incubating salmon muscle tissue with root extracts of CM collected from urban areas, where there was also a statistically no-significant increase in SOD activity (by11.5%, p >0.05) compared to the control samples (394.19 ±20.75 U.mg^{-1} protein vs. 353.5 ±16.69 U.mg^{-1} protein). After incubation of salmon muscle tissue with stalk extracts of CM collected from urban areas, we noted an increase in superoxide dismutase activity by 7.2% (p >0.05) compared to the control samples (378.86 ±44.94 U.mg^{-1} protein vs. 353.5 ±16.69 U.mg^{-1} protein). Also, we recorded a statistically no-significant elevation of SOD activity in muscle tissue incubated *in vitro* with root extracts of CM harvested from rural areas (by 5.6%, p >0.05) compared to the control samples (373.43 ±22.02 U.mg^{-1} protein vs. 353.5 ±16.69 U.mg^{-1} protein) (Figure 4).

The catalase activity in the muscle tissue of Atlantic salmon after *in vitro* incubation with extracts derived from roots and stalks of CM collected from rural and urban areas was presented in Figure 5.

Analyzing catalase activity after the incubation of muscle tissue with CM extracts, we obtained interesting results. We noted a statistically significant increase in catalase activity after *in vitro* incubation of salmon muscle tissue with root extracts of CM collected from urban areas (by 11.5%, p <0.05) compared to controls (394.19 ±20.75 µmol.min^{-1}.mg^{-1} protein vs. 353.5 ±16.69 µmol.min^{-1}.mg^{-1} protein). Similar results were obtained after incubating muscle tissue with stalk extracts of CM collected from rural agglomerations, where there was also a statistically significant increase in CAT activity by 12.8% (p <0.05) compared to the control samples (398.68 ±23.38 µmol.min^{-1}.mg^{-1} protein vs. 353.5 ±16.69 µmol.min^{-1}.mg^{-1} protein). A statistically no-significant elevation in catalase activity was observed after incubating salmon muscle tissue with stalk extracts of CM collected from urban areas (378.86 ±44.94 µmol.min^{-1}.mg^{-1} protein vs. 353.5 ±16.69 µmol.min^{-1}.mg^{-1} protein) and root extracts of CM collected from rural areas (373.43 ±22.02 µmol.min^{-1}.mg^{-1} protein vs. 353.5 ±16.69 µmol.min^{-1}.mg^{-1}

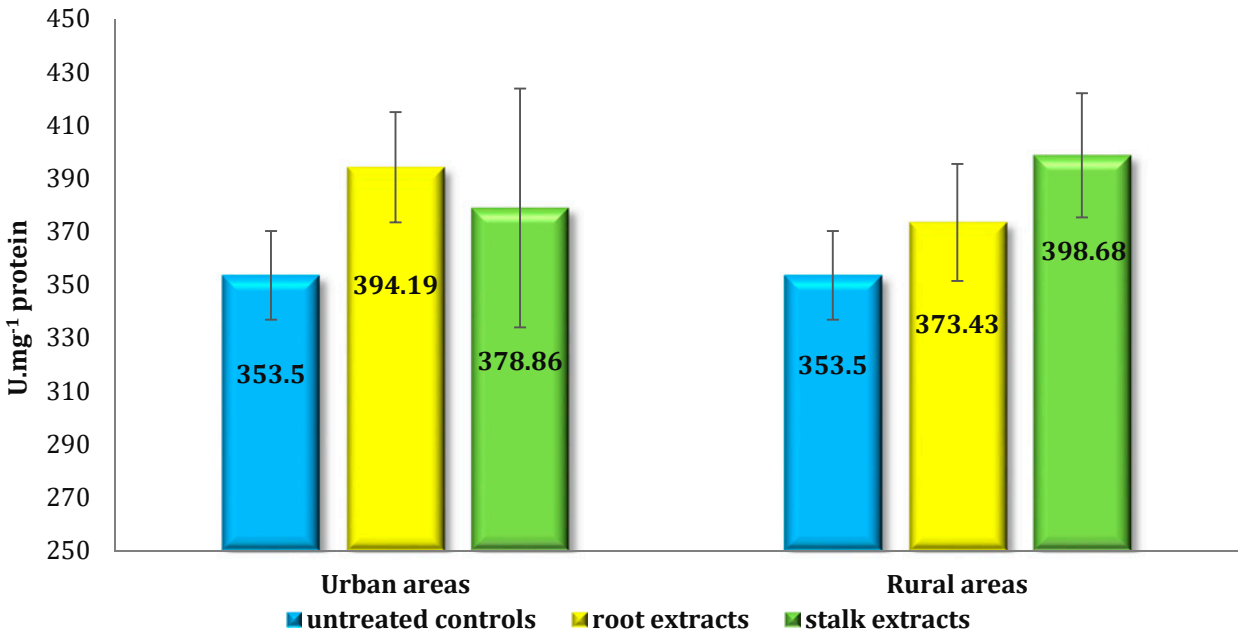

Figure 4 The superoxide dismutase activity in the muscle tissue of Atlantic salmon after *in vitro* incubation with root and stalk extracts of *Chelidonium majus* L. collected from rural and urban areas of the Pomeranian region (M ±m, n = 8)

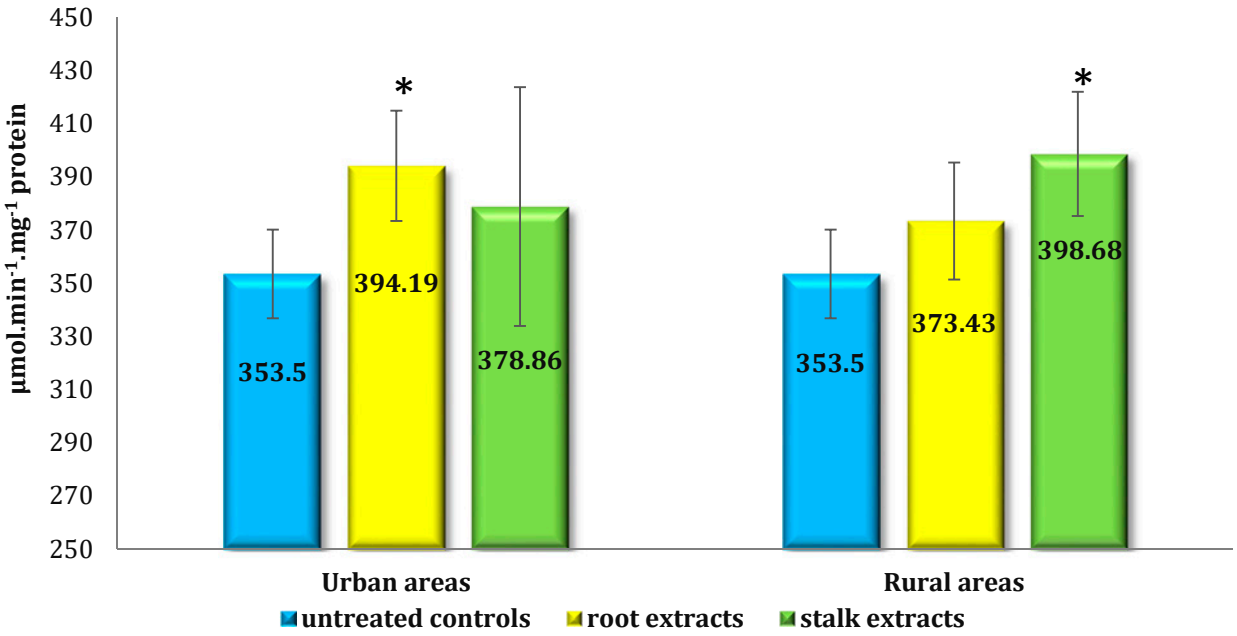

Figure 5 The catalase activity in the muscle tissue of Atlantic salmon after *in vitro* incubation with root and stalk extracts of *Chelidonium majus* L. collected from rural and urban areas of the Pomeranian region(M ±m, n = 8)
*– statistically significant differences (p <0.05) compared to the control samples

protein). There was an increase in CAT activity by 7.2% (p >0.05) and 5.6% (p >0.05), respectively compared to control samples (Figure 5).

The glutathione peroxidase activity in the muscle tissue of Atlantic salmon after *in vitro* incubation with extracts derived from roots and stalks of CM collected from rural and urban areas was presented in Figure 6.

We observed a statistically significant reduction in the GPx activity in salmon muscle tissue after treatment with root extracts of CM harvested from both urban and rural areas compared to the control samples (190.99 ±6.47 nmol.min^{-1}.mg^{-1} protein and 188.24 ±5.26 nmol.min^{-1}.mg^{-1} protein vs. 218.89 ±7.64 nmol.min^{-1}.mg^{-1} protein, respectively). There

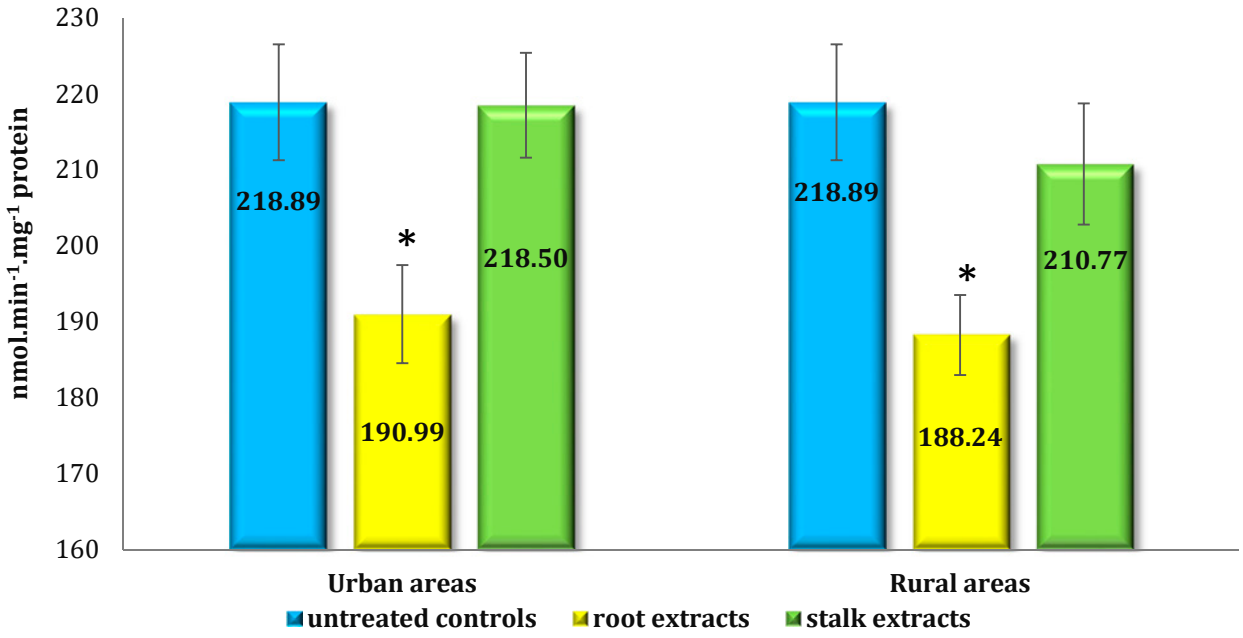

Figure 6 The glutathione peroxidase activity in the muscle tissue of Atlantic salmon after *in vitro* incubation with root and stalk extracts of *Chelidonium majus* L. collected from rural and urban areas of Pomeranian region (M ±m, n = 8)
*– statistically significant differences (p <0.05) compared to the control samples

was a decrease in GPx activity by 12.7% ($p < 0.05$) and 14% ($p < 0.05$), respectively. After *in vitro* incubation of salmon muscle tissue with stalk extracts of CM collected from both urban (218.5 ±6.9 $nmol.min^{-1}.mg^{-1}$ protein) and rural areas (210.77 ±7.98 $nmol.min^{-1}.mg^{-1}$ protein), we recorded a decrease in GPx activity, but not statistically significantly (by 0.2%, $p > 0.05$ and 3.71%, $p > 0.05$, respectively) compared to the control samples (Figure 6).

In the current study, we investigated the effects of CM extracts on lipid peroxidation and biomarkers of oxidatively modified proteins, as well as on antioxidant defence in the muscle tissue of Atlantic salmon. Our study suggests that the extracts from both roots and stems of CM harvested from urban areas reduced the level of oxidatively modified proteins. Analyzing the total antioxidant capacity after the incubation with CM extracts under *in vitro* conditions, we concluded that extracts derived mainly from the stalks of CM harvested from both urban and rural areas effectively increase TAC levels. These results are reflected after analysis of antioxidant enzyme activity, where we observed statistically significant increases in the activity of superoxide dismutase and catalase. This may be related to the presence of antioxidant compounds contained in the plant structures of greater celandine.

In our previous study (Stefanowski et al., 2021a, c, d) on muscle tissue of rainbow trout (*Oncorhynchus mykiss* Walbum), we also demonstrated the antioxidant activity of CM extracts. Our results showed that extracts of CM collected from both urban and rural areas statistically significantly reduced the level of aldehyde derivatives of OMB by 18.8% ($p < 0.05$). The analysis of the levels of ketonic derivatives of OMP showed that extracts of CM collected from both urban and rural areas statistically significantly decreased the level of ketonic derivatives of OMP by 20.6 and 21.5%, respectively (for urban areas), as well as 26.7 and 12.5% (for rural areas). Lower levels of lipid peroxidation were observed after incubation with stalk extracts, while those collected from rural areas showed the lowest result (by 11%). Root extracts of CM collected from urban and rural areas increased TBARS levels. Analysis of oxidatively modified protein levels in the blood of rainbow trout after *in vitro* incubation with root and stem extracts shows that extracts can inhibit the production of oxidative carbonyls by scavenging free radicals (Stefanowski et al., 2021c, d).

In another study (Stefanowski et al., 2022) on muscle tissue of rainbow trout (*Oncorhynchus mykiss* Walbum), we also demonstrated the dose-dependent antioxidant activity of CM extracts. Results of our study revealed that a final dose of CM extracts of 0.63 $mg.mL^{-1}$ showed the highest antioxidant activity in the muscle tissue of rainbow trout. The extracts derived mainly from the roots of CM collected from rural areas were effective in reducing the levels of oxidative stress biomarkers by reducing lipid peroxidation markers, which may suggest that the active substances such as alkaloids (chelidonine, sanguinarine, berberine), flavonoids, phenols in these plants can effectively protect the membrane structures in muscle cells of salmonids. We also observed statistically significant reductions in levels of both aldehydic and ketonic derivatives of oxidatively modified proteins in the muscle tissue of rainbow trout after incubation with CM extracts at this dose compared to the controls. The comparison of these results showed that CM extracts can effectively inhibit protein damage by scavenging free radicals and acting on antioxidant defences. The secondary metabolites of CM, i.e. polyphenols and alkaloids, are most likely responsible for this effect. Using extracts in final doses of 5 $mg.mL^{-1}$, 2.5 $mg.mL^{-1}$, and 1.25 $mg.mL^{-1}$ derived from both roots and stalks resulted in statistically significant increases in levels of TBARS and OMP (Stefanowski et al., 2022).

Pharmacologically relevant substances of CM are isoquinoline alkaloids. Generally, five groups of alkaloids were found in CM. These are the derivatives of phenanthridine (3,4-benzoisoquinoline), protoberberine, protopine, quinolizidine, and aporphine. Major phenanthridine derivatives that were found in aerial and underground parts are chelidonine and chelerythrine (Zielińska et al., 2018). Nile et al. (2021) have studied total phenolics and flavonoids in the different parts of CM. The leaves showed higher flavonoid content (137.43 $mg.g^{-1}$), while the pod showed the highest phenolic (23.67 $mg.g^{-1}$) content when compared with the stems, flowers, and roots. In the ABTS (Diammonium 2,2'-azinobis[3-ethyl-2,3-dihydrobenzothiazole-6-sulphonate]) antioxidant assay, the flower extract showed a 57.94% effect, while the leaf, pod, and root extract exhibited 39.10%, 36.08%, and 28.88% activity, respectively. The pod and leaf extracts demonstrated the potential effect, exhibiting 45.46 and 41.61% activity, respectively, for the DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay. Similar to the phosphomolybdenum assay, the flower revealed higher antioxidant activity (46.82%) than the other plant parts (Nile et al., 2021).

Some of the versatile traditional uses of CM can be explained, as in many other herbs, by anti-inflammatory potential targeting various pathways in the organism

as well as modulation of the immune response. Both have been confirmed in many studies using *in vitro* cellular models, as well as *in vivo*. The ability to inhibit inflammation or, in some cases, to stimulate immune response and mitigate excessive reactiveness can contribute to the postulated anticancer properties and improve symptoms of gastric disorders as well. Chelidonic acid was efficient in mouse models of ovalbumin-elicited allergic rhinitis (Oh et al., 2011) and ulcerative colitis (Kim et al., 2012). This compound also attenuated inflammatory responses by reducing levels and gene expression of several mediators and enzymes in colon tissues (cyclooxygenase-2, Hypoxia-inducible factor 1-α, prostaglandin E2) and in allergic mice (Interleukin (IL)-4, IL-1β, cyclooxygenase-2, caspase-1, and increase of interferon-γ). In the human mast cell line HMC-1 stimulated for inflammatory response by the phorbol ester (TPA) and calcium ionophore A23187, chelidonic acid inhibited IL-6 expression by blocking nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) (Shin et al., 2011).

Most authors (Lee et al., 2007; Yang et al., 2011) using different experimental models of inflammation *in vitro* demonstrated the anti-inflammatory activity of CM extracts. It was found that mainly alkaloids contained in extracts may be responsible for these anti-inflammatory effects. The analysis of Nawrot et al. (2007a, b) confirmed the presence of the protein components of the antioxidant defence system in CM latex. These proteins form the first line of defence against different stress conditions and help to prevent the attack of different pathogens, which are highly abundant in the milky sap. Peroxidase 12-like and isoflavone reductase homolog were present only in the milky sap (Nawrot et al., 2007a, b).

Zielińska et al. (2020) used the LC-MS/MS method to determine alkaloids, phenolic acids, carboxylic acids, and hydroxybenzoic acids in the CM extracts. These researchers investigated five individually tested alkaloids (coptisine, berberine, chelidonine, chelerythrine, and sanguinarine) as well as CM root extract for their effect on the secretion of interleukins (IL-1β, IL-8), and tumor necrosis factor α (TNF-α) in human polymorphonuclear leukocytes (neutrophils). Berberine, chelidonine, and chelerythrine significantly decreased the secretion of TNF-α in a concentration-dependent manner. Sanguinarine was the most potent inhibitor of IL-1β secretion. However, the overproduction of IL-8 and TNF-α and high cytotoxicity for these compounds were observed. Coptisine was highly cytotoxic and slightly decreased the secretion of the studied cytokines. According to Zielińska et al. (2020), the extract (1.25–12.5 $\mu g.mL^{-1}$) increased cytokine secretion in a concentration-dependent manner, but an increase in cytotoxicity was also noted.

Park et al. (2015) investigated the effects of CM extract on human epidermoid carcinoma A431 cells through multiple mechanisms, including induction of cell cycle arrest, activation of the caspase-dependent pathway, blocking of nuclear factor-κB (NF-κB) activation and involvement in the mitogen-activated protein kinase (MAPK) pathway. CM inhibited the proliferation of A431 cells in a dose- and time-dependent manner, increased the percentage of apoptotic cells, significantly decreased the mRNA levels of cyclin D1, Bcl-2, Mcl-1, and survivin, as well as increased p21 and Bax expression. Exposure of A431 cells to CM extract enhanced the activities of caspase-3 and caspase-9, while co-treatment with CM, the pan-caspase inhibitor Z-VAD-FMK, and the caspase-3 inhibitor, Z-DEVE-FMK, increased the proliferation of A431 cells. CM extract not only inhibited NF-κB activation, but it also activated p38 MAPK and MEK/ERK signaling. These results demonstrated that CM extract inhibited the proliferation of human epidermoid carcinoma A431 cells by inducing apoptosis through caspase activation and NF-κB inhibition *via* a MAPK-independent pathway (Park et al., 2015).

Chelidonine is known for its broad pharmacological activities that lead to anti-inflammation, anti-viral and anti-cancer effects. To be specific, chelidonine treatment induced apoptosis in T98G glioma cells, MCF-7 and SK-BR-3 breast adenocarcinoma, HepG2 hepatoma, HeLa cervical cancer, SW620 colon cancer, head and neck squamous cell carcinoma HNSCC, human gastric carcinoma SGC-7901, and leukaemia MT-4 cells, through caspase, cell cycle checkpoints, and MAP kinase pathways. In colon cancer, Caco-2, and leukaemia cell line CEM/ADR 5000, metabolic enzyme regulation by chelidonine reversed doxorubicin resistance. Chelidonine was also reported to trigger autophagy, cellular senescence, and blocking telomerase activity. The cytotoxic effect of chelidonine and its mechanisms on pancreatic cancer have not been elucidated (Paul et al., 2012; Noureini and Esmaili, 2014).

According to Orvos et al. (2015), hydroalcoholic extracts of greater celandine and its alkaloids, especially berberine, chelidonine, and sanguinarine have a significant hERG potassium channel-blocking effect. These extracts and alkaloids also prolong the cardiac action potential in dog ventricular muscle. Therefore these compounds may consequently delay cardiac repolarization, which may result in the prolongation

of the QT interval and increase the risk of potentially fatal ventricular arrhythmias.

Shen et al. (2022) suggested a potential therapeutic role of CM against ovarian cancer due to induced SKOV-3 cell death by increasing levels of activating transcription factor 3 (ATF3) and its downstream proteins Tip60 and Foxo3a. CM upregulated the expression of ATF3 and tightly regulated transcriptional regulator (Tip60) and promoted Foxo3a nuclear translocation, ultimately increasing the level of the Bcl-2-associated X protein (Bax) protein. ATF3 overexpression stimulated Tip60 expression, while ATF3 inhibition by siRNA repressed Tip60 expression. Furthermore, siRNA-mediated Tip60 inhibition significantly promoted Foxo3a phosphorylation, leading to a blockade of Foxo3a translocation into the nucleus. Thus, ATF3 mediates the regulation of Foxo3a by Tip60. Moreover, siRNA-mediated Foxo3a inhibition suppressed the expression of Bax and subsequent apoptosis (Shen et al., 2022).

Conclusions

The results revealed that the incubation of salmon muscle tissue with extracts derived from both the stems and roots of CM harvested from rural areas resulted in a decrease in lipid peroxidation. Similarly, the use of extracts derived from the roots of CM collected from urban areas resulted in a decrease in TBARS levels. These results suggest that it can be argued that the presence of secondary plant metabolites in CM extracts protects structures of cell membranes against the damaging effects of free radicals. On the other hand, analysis of levels of protein oxidation after incubation of muscle tissue with CM extracts showed that extracts derived from both roots and stalks of CM harvested from urban areas reduced levels of ketonic derivatives of oxidatively modified proteins. Analyzing the total antioxidant capacity after the incubation with CM extracts under *in vitro* conditions, we concluded that extracts mainly derived from the stalks of CM harvested from both urban and rural areas effectively increase TAC levels. These results are reflected after analysis of antioxidant enzyme activity, where we observed statistically significant increases in superoxide dismutase and catalase activity. On the other hand, the incubation of CM extracts with muscle tissue resulted in a statistically significant decrease in glutathione peroxidase activity compared to the control samples.

Conflict of interests

The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

Acknowledgements

This research was supported by the Pomeranian University in Słupsk (Poland).

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

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Polyphenol compounds and antioxidant activity of *Salvia officinalis* L. and *Salvia sclarea* L.

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Article Details:
Received:2022-07-04
Accepted:2022-08-01
Available online:2022-11-30

DOI: https://doi.org/10.15414/ainhlq.2022.0015

The plants from *Salvia* L. (sage) genus are well-known as culinary, ornamental, aromatic, and medicine plants widely distributed in the world. The plant raw of these plants contains numerous biologically active compounds that determine the different biological activities. This study aimed to evaluate the antioxidant activity and polyphenol content of ethanol extracts of *Salvia officinalis* L. and *S. sclarea* L. during vegetation. Plant raw material was collected from an experimental collection of aromatic and medicinal plants of the Institute of Climate Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine (Kherson region, v. Plodove) in 2020–2021. It investigated polyphenol, phenolic acid, flavonoid content, molybdenum-reducing power of extracts, and radical scavenging activity by the DPPH method. The total polyphenol compound content was 24.52–95.62 mg GAE.g^{-1} (mg gallic acid equivalent per gram) for *S. officinalis* and 29.39–91.02 mg GAE.g^{-1} for *S. sclarea*. The total phenolic acid content of *S. officinalis* and *S. sclarea* extracts was 10.18–40.23 and 10.13–36.01 mg CAE.g^{-1} (mg caffeic acid equivalent per gram), respectively. The total flavonoid content was from 13.73 to 55.38 mg QE.g^{-1} (quercetin equivalen per gram) for *S. officinalis* and from 25.91 to 53.82 mg QE.g^{-1}for *S. sclarea*. The free radical scavenging activity of ethanol extracts of *S. officinalis* and *S. sclarea* was 6.05–8.59 mg TE.g^{-1} (mg Trolox equivalent per gram) and 6.56–8.03 mg TE.g^{-1}, respectively. The extracts of *S. officinalis* showed molybdenum-reducing power from 56.25 to 218.67 mg TE.g^{-1} and *S. sclarea* extracts from 35.42 to 162.65 mg TE.g^{-1}. A strong correlation was found between free radical scavenging activity and polyphenol compound groups (r = 0.723–0.868), and between molybdenum-reducing power and polyphenol compound groups (r = 0.759–0.927) for *S. officinalis*. Strong relations were found in extracts of *S. sclarea* between molybdenum-reducing power and investigated polyphenol compounds (r = 0.802–0.909), whereas, free radical scavenging activity weak correlated with investigated compounds. Thus, this study showed that *S. officinalis* and *S. sclarea* extracts are a source of antioxidant compounds that can be used in the pharmaceutical and food industries. Most content of antioxidant compounds is found in the leaf and inflorescence extracts.

Keywords: sage, polyphenols, flavonoids, phenolic acids, correlation

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Introduction

The species from *Salvia* L. (sage) genus are well-known ornamental, aromatic, and medicine plants widely distributed in European countries, and their number achieved 500 (Korablova et al., 2019). It's one of the most species-rich genera and the exact number of species is still being determined (González-Gallegos et al., 2020). These species were *domestica*ted more than 3,500 years BC, used as food plants in some countries, and are perspective nutraceutical crops (Sosa et al., 2016). These are perennial and annual plants, woody shrubs, and habitats related to seed dispersal and can be from desert to dry shrubland, etc. (Zona, 2017). Many species from this genus are used as culinary herbs in different countries (Hamidpour et al., 2014) and used in pharmaceutical and cosmetic industries (Grdiša et al., 2015).

As aromatic cultures, plants *S. officinalis* and *S. sclarea* are characterized by the rich content of essential oil with numerous biological activity compounds (Vergine et al., 2019; Ovidi et al., 2021). The essential oil composition of these species is α-pinene, camphene, β-pinene, *p*-cymene, 1,8-cineole, γ-terpinene, α-thujone, chrysanthenone, camphore, etc. (Ovidi et al., 2021).

The biochemical composition of plant raw of *S. officinalis* L. is terpenoids, flavonoids (Topçu, 2006; Topcu and Kusman, 2014), polyphenols, flavones, proteins, reducing sugars (Neagu et al., 2014), polyunsaturated acids (Sosa et al., 2016), phenolic acids (Paje et al., 2022). Seeds of some species id a good source of α-linolenic acid (Nitrayová et al., 2014). Different extracts of *Salvia* species exhibited various biological activities such as antioxidant, anti-cancer, anti-diabetic, anti-diarrheal, decreasing of cholesterol levels, improving memory (Hamidpour et al., 2014), antimicrobial, and anti-inflammatory (Sharopov et al., 2018).

The pharmacological activities of phenolic acids of *Salvia* species are anti-oxygenation, antithrombotic, anti-liver injury activity, anti-tumour, anti-hypertensive effect, antiviral, etc. (Wang et al., 2019). The comparable analysis of *S. officinalis* and *S. sclarea* antioxidant activity showed that oil and seeds of the first species exhibited the highest values (Živković et al., 2017). Extracts of *S. officinalis* and *S. sclarea* were effective against *E. coli*, *P. fluorescens*, *A. bohemicus*, *K. marina*, and *B. cereus* by disc diffusion method (Ovidi et al., 2021).

This study aimed to estimate the polyphenol content and antioxidant activity of two *Salvia* species in the South region of Ukraine as a potential source of antioxidants that can be used in further pharmacological research.

Material and methodology

Biological material

The plants of *Salvia officinalis* L. and *S. sclarea* L. (Figure 1) were investigated in this study from the experimental collection of the Institute of Climate Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine (Kherson region, v. Plodove) in 2020–2021. The plant raw took at the budding (buds, leaves, and all above-ground part), flowering (leaves, inflorescences, and all above-ground part), and fruitage (leaves, fruits, and all above-ground part).

All biochemical analyses were conducted at the Slovak University of Agriculture in Nitra (Slovak Republic).

Figure 1 Plants of *Salvia officinalis* L. (1) and *S. sclarea* L. (2) at the flowering stage

Chemicals

All chemicals used were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA) and CentralChem (Slovakia).

Preparations of extracts

An amount of 0.25 g of each sample was extracted with 20 mL of 80% ethanol for 2 h in a laboratory shaker GFL 3005 (GFL, Burgwedel, Germany). Then, the samples were centrifuged at 4605 RCF (Rotofix 32 A, Hettich, Germany) for 10 min and the supernatant was used for measurement of FRSA (antiradical activity) using DPPH, MRAP (antioxidant activity) using phosphomolybdenum method and measurement of other antioxidant properties (detection of total polyphenol, total flavonoid, and phenolic acid content).

Total polyphenol content of extracts

The total polyphenol content (TPC) was measured by the method of Singleton and Rossi (1965) using the Folin-Ciocalteu reagent. A quantity of 0.1 mL of each sample was mixed with 0.1 mL of the Folin-Ciocalteu reagent, 1 mL of 20% (w/v) sodium carbonate, and 8.8 mL of distilled water. After 30 min in darkness, the absorbance at 700 nm was measured with the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25–300 $mg.L^{-1}$; R^2 = 0.998) was used as the standard. The results were expressed in $mg.g^{-1}$ DW gallic acid equivalent.

Total phenolic acid content

The content of phenolic acids was determined using Farmakopea Polska (1999). 0.5 ml of sample extract was mixed with 0.5 ml of 0.5 M hydrochloric acid, 0.5 ml Arnova reagent, 0.5 ml of 1 M sodium hydroxide (w/v), and 0.5 ml of distilled water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid 1–200 $mg.l^{-1}$ (R^2 = 0.999) was used as a standard. The results were expressed in $mg.g^{-1}$ caffeic acid equivalents (CAE).

Total flavonoid content of extracts

The total flavonoid content (TFC) was determined by the modified method described by Shafii et al. (2017). An aliquot of 0.5 mL of the sample was mixed with 0.1 mL of 10% (w/v) ethanolic solution of aluminium chloride, 0.1 mL of 1 M potassium acetate, and 4.3 mL of distilled water. After 30 min in darkness, the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (1–400 $mg.L^{-1}$; R^2 = 0.9977) was used as the standard. The results were expressed in $mg.g^{-1}$ DW quercetin equivalent.

Free radical scavenging activity

Free radical scavenging activity (FRSA) of samples (antiradical activity) was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sánchéz-Moreno et al., 1998). An amount of 0.4 mL of sample was mixed with 3.6 mL of DPPH solution (0.025 g DPPH in 100 mL ethanol). The absorbance of the reaction mixture was determined with the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10–100 $mg.L^{-1}$; R^2 = 0.989) was used as the standard and the results were expressed in $mg.g^{-1}$ DM Trolox equivalents.

Molybdenum-reducing power of extracts

The molybdenum-reducing power (MRP) of samples was determined by the method of Prieto et al. (1999) with slight modifications. The mixture of the sample (1 mL), monopotassium phosphate (2.8 mL, 0.1 M), sulfuric acid (6 mL, 1 M), ammonium heptamolybdate (0.4 mL, 0.1 M), and distilled water (0.8 mL) was incubated at 90 °C for 120 min, then cooled to room temperature. The absorbance at 700 nm was detected with the spectrophotometer Jenway (6405 UV/Vis, England). Trolox (10–1000 $mg.L^{-1}$; R^2 = 0.998) was used as the standard and the results were expressed in $mg.g^{-1}$ DM Trolox equivalent.

Statistical analysis

The results are expressed as mean values of three replications ± standard deviation (SD); hierarchical cluster analyses of similarity between samples were computed based on the Euclidean similarity index. Data were analyzed with the ANOVA test and differences between means were compared through the Tukey-Kramer test ($p < 0.05$).

Results and discussions

The study of antioxidant activity is one of the distributed topics of the last decades and is related to phenolic substances (Nićiforović et al., 2010). Polyphenol compounds are a group of antioxidants that includes flavonoids, phenolic acids, and their subclasses that are found in teas, fruits, juices, wines, olive oil, and chocolates. They have synthetic, medicinal, and industrial value (Handique and Baruah, 2002; Perron and Brumaghim, 2009). Polyphenol compounds are interesting as anti-inflammatory, anti-cancer, and

anti-ageing agents, widely used for cosmetic and nutraceutical purposes (Munin and Edwards-Lévy, 2011). Polyphenol compounds of *Salvia* species include rosmarinic acid, vanillin, chlorogenic acid, catechin, quercetin, and p-coumaric acid (Rowsan and Najafian, 2020).

The content of polyphenol compounds of ethanol extracts of *S. officinalis* and *S. sclarea* was from 24.52 (*S. officinalis*, leaves at the budding) to 95.62 (*S. officinalis*, above-ground parts at the fruitage) mg GAE.g^{-1} (Figure 2). The polyphenol compound content of *S. officinalis* raw was 24.25–87.36 mg GAE.g^{-1} during vegetation. The different parts of *S. sclarea* demonstrated the polyphenol content from 29.39 to 91.02 mg GAE.g^{-1}. The content of these compounds in the herb (all above-ground parts) was minimal at the budding stage and fruitage for both species, whereas minimal polyphenol content was only in *S. sclarea* raw of herb at the flowering stage. The above-ground part of *S. officinalis* at the flowering stage demonstrated the highest content of polyphenols than other parts at this period.

The study of eight *Salvia* species showed that total polyphenol content was in the range of 50.3–167.1 mg GAE.g^{-1} depending on the species (Tosun et al., 2009). The comparative study of Iranian *Salvia* species showed that the total polyphenol content was from 38 to 326 mg GAE.g^{-1} (Asadi et al., 2010). According to Jasicka-Misiak et al. (2018), the total content of polyphenols of *S. officinalis* and *S. sclarea* extracts was 63.9–93.8 mg GAE.g^{-1} and 96.1–134.4 mg GAE.g^{-1}, respectively. According to Afonso et al. (2019), the total polyphenol content in water extracts of *S. mexicana*, *S. officinalis*, and *S. africana* was 158, 229, and 350.6 µg GAE.mg^{-1} extracts. The study of three *Salvia* species showed that methanol extracts had a polyphenol content of 658.3–1805.9 mg caffeic acid equivalent per 100 g FW (fresh weight) (Sharopov et al., 2018). According to Mňahončaková et al. (2019), the polyphenol content in an extract of Slovakian *S. officinalis* was 62.87 mg GAE.g^{-1} at the stage of flowering was less compared with our study (87.36 mg GAE.g^{-1}). The polyphenol content of another Lamiaceae species *Scutellaria baicalensis* Georgi from the same region of Ukraine (Kherson area) was 96.54 mg GAE.g^{-1} (Vergun et al., 2019). Also, the study of *Thymus* herb demonstrated that total polyphenol content varied from 56.12 to 98.36 mg GAE.g^{-1} depending on species at the flowering stage (Vergun et al., 2022).

A phenolic acid is a group of phenolic compounds that play an important role as an antiaging agent, and demonstrate antitumor, antimicrobial, and anti-inflammatory properties. These biologically active molecules are found in edible and nonedible plants (Jitan et al., 2018). As reported Wang et al. (2019), phenolic acids are the main active polyphenol compounds of *Salvia* species with high therapeutic functions, among which caffeic acid and danshensu are structural units.

The total content of phenolic acids in the ethanol extracts was from 10.13 to 40.23 mg CAE.g^{-1} during vegetation depending on species (Figure 3). The minimal content

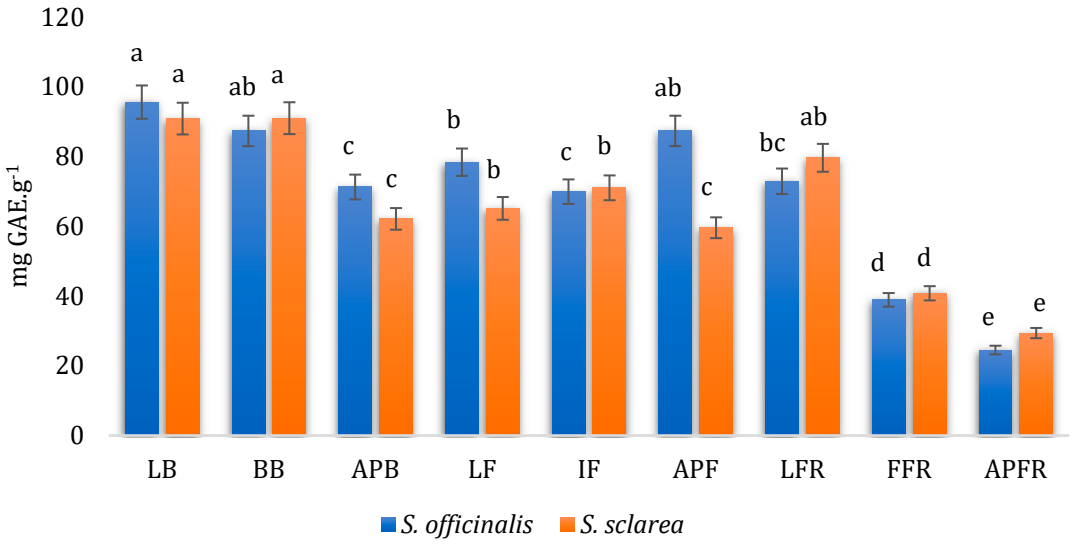

Figure 2 The content of polyphenol compounds in ethanol extracts of *Salvia officinalis* L. and *S. sclarea* L.
GAE – gallic acid equivalent; LB – leaves at the budding; BB – buds at the budding; APB – above-ground part of the plant at the budding stage; LF – leaves at the flowering; IF – inflorescences at the flowering stage; APF – above-ground part of the plant at the flowering stage; LFR – leaves at the fruitage; FFR – fruits at the fruitage; APFR – above-ground part of the plant at the fruitage. Different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

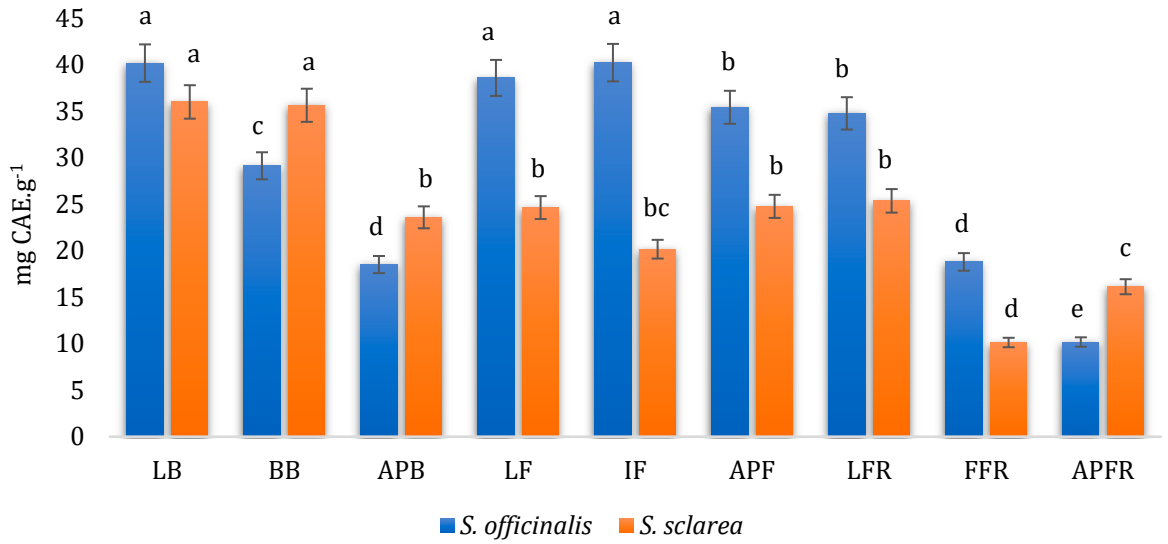

Figure 3 The content of phenolic acids in ethanol extracts of *Salvia officinalis* L. and *S. sclarea* L.
CAE – caffeic acid equivalent; LB – leaves at the budding; BB – buds at the budding; APB – above-ground part of the plant at the budding stage; LF – leaves at the flowering; IF – inflorescences at the flowering stage; APF – above-ground part of the plant at the flowering stage; LFR – leaves at the fruitage; FFR – fruits at the fruitage; APFR – above-ground part of the plant at the fruitage. Different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

of phenolic acids for both species was determined at the budding stage in the herb. The maximal values of phenolic acids at the flowering stage were determined for *S. officinalis* raw. The highest values of these compounds of *S. officinalis* extracts were found at the budding and fruitage. The phenolic acids in *S. sclarea* extracts accumulated unevenly.

As reported Orhan et al. (2012), the extracts of Turkish species of *Salvia* weren't identified as gallic acid. According to Mňahončaková et al. (2019), the phenolic acid content in *S. officinalis* extracts was 24.30 mg CAE.g^{-1}. The phenolic acid content of *Scutellaria baicalensis* from the same region of Ukraine was 30.12 mg CAE.g^{-1} (Vergun et al., 2019). The most abundant phenolic acid of *Salvia* species is rosmarinic acid (Paje et al., 2022). Extracts of *Thymus* species demonstrated from 26.19 to 40.46 mg CAE.g^{-1} depending on species at the flowering stage which is close to the results in this study (Vergun et al., 2022).

Flavonoids are a versatile class of natural compounds that demonstrated different biological activities such as antimicrobial and antifungal (Saleem et al., 2018). These polyphenol compounds are abundant in fruits, vegetables, and grains, and have antioxidant, anti-inflammatory activity and reduce the risk of diseases (Shen et al., 2022). Flavonoids from some *Salvia* species had α-amylase and α-glucosidase inhibitory effect that has an important antidiabetic effect (Asghari et al., 2015).

The content of flavonoids in ethanol extracts of investigated *Salvia* species was from 13.73 to 55.38 mg QE.g^{-1} during the flowering period depending on the species (Figure 4). The content of flavonoids in raw of *S. officinalis* and *S. sclarea* was 13.73–55.38 and 25.91–53.82 mg QE.g^{-1}, respectively. The highest content of flavonoids of *S. officinalis* raw accumulated in leaf extracts during vegetation, whereas *S. sclarea* accumulated these compounds in the leaves at the budding and fruitage. At the flowering stage, flavonoid content was maximal in the inflorescences extracts of *S. sclarea*.

As reported Asadi et al. (2010), the total flavonoid content in extracts of Iranian species was from 91 to 253 mg of catechin per gram extracts. According to Sharopov et al. (2018), the flavonoid content of methanol extracts of these species varied from 13 to 184.9 mg QE.100 g^{-1} FW. According to Mňahončaková et al. (2019), the flavonoid content in extracts of *S. officinalis* growing in Slovakia was 36.80 mg QE.g^{-1}. The flavonoid content of *Scutellaria baicalensis* from the same region of Ukraine was 66.07 mg QE.g^{-1} (Vergun et al., 2019) which was higher than in extracts of investigated plants of *Salvia*. A similar content of flavonoids was found for *Thymus* species at the flowering stage and was 24.59–49.29 mg QE.g^{-1} (Vergun et al., 2022).

Antioxidants play an essential role in living organisms on a cellular level and this is important to search for new natural sources of these compounds (Lourenço et al., 2019). The value of the antioxidant activity of

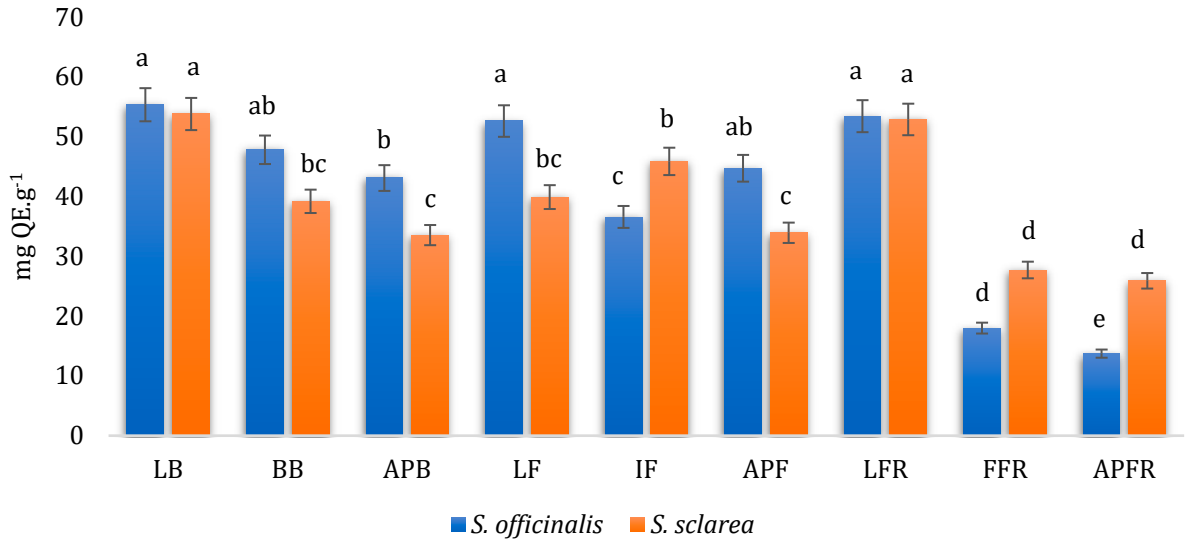

Figure 4 The content of flavonoids in ethanol extracts of *Salvia officinalis* L. and *S. sclarea* L.
QE – quercetin equivalent; LB – leaves at the budding; BB – buds at the budding; APB – above-ground part of the plant at the budding stage; LF – leaves at the flowering; IF – inflorescences at the flowering stage; APF – above-ground part of the plant at the flowering stage; LFR – leaves at the fruitage; FFR – fruits at the fruitage; APFR – above-ground part of the plant at the fruitage. Different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

plant raw depends on many factors among which are a method of determination and the type of solvent (Dawidowicz et al., 2012). As reported Francik et al. (2020), extracts of *S. officinalis* leaves, for example, are characterized by higher polyphenol content than infusions. One of the most popular methods is DPPH (radical scavenging activity) which is widely used to determine antioxidants with a polyphenol nature (Marinova and Batchvarov, 2011).

Numerous studies demonstrated that *Salvia* extracts had significant antioxidant activity by different methods among which DPPH, FRAP, TEAC, and ABTS (Asadi et al., 2010; Kačmárová et al., 2016).

The antioxidant activity of the DPPH method was from 6.97 to 8.14 mg TE.g^{-1} at the start of vegetation and from 4.6 to 6.69 mg TE.g^{-1} at the flowering stage (Figure 5). The maximal values of antioxidant activity by the DPPH

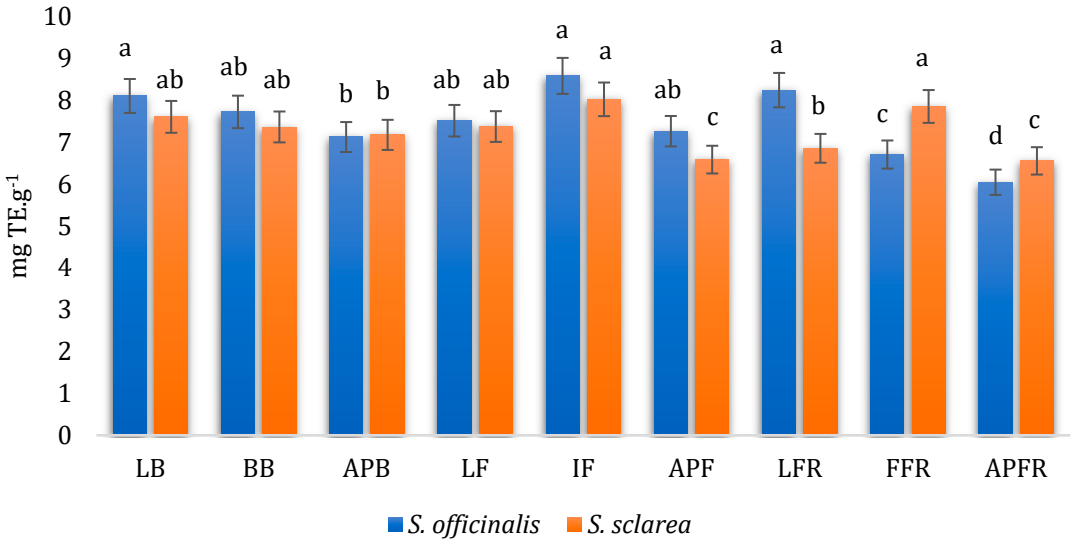

Figure 5 Free radical scavenging activity of ethanol extracts of *Salvia officinalis* L. and *S. sclarea* L.
TE – Trolox equivalent; LB – leaves at the budding; BB – buds at the budding; APB – above-ground part of the plant at the budding stage; LF – leaves at the flowering; IF – inflorescences at the flowering stage; APF – above-ground part of the plant at the flowering stage; LFR – leaves at the fruitage; FFR – fruits at the fruitage; APFR – above-ground part of the plant at the fruitage. Different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

method were found for leaf, inflorescences, and fruit extracts.

According to Mňahončaková et al. (2019), the free radical scavenging activity of *S. officinalis* extracts was 7.78 mg TE.g^{-1}. *Thymus* extracts of plants at the flowering stage exhibited free radical scavenging activity from 8.0 to 8.74 mg TE.g^{-1} (Vergun et al., 2022).

Among the numerous methods of antioxidant activity assessment also well-known molybdenum-reducing power of extracts that are based on reducing molybdenum by anti-oxygen agents (Vasyliev et al., 2020). The antioxidant activity by the phosphomolybdenum method of investigated plants was from 56.25 to 218.67 mg TE.g^{-1} for *S. officinalis* and from 35.42 to 162.65 mg TE.g^{-1} depending on species and part of plants (Figure 6). The highest molybdenum-reducing power was found for leaves of *S. officinalis* extracts during vegetation. The extracts of *S. sclarea* demonstrated maximal values of this parameter in the leaves at the budding and fruitage and herbs at the flowering stage.

According to Mňahončaková et al. (2019), the molybdenum-reducing power of *S. officinalis* extracts was 174.50 mg TE.g^{-1}. This parameter was varied in extracts of *Scutellaria baicalensis* from the same region of Ukraine (63.33–260.24 mg TE.g^{-1}) but above-ground parts demonstrated the highest reducing power of extracts compared with separate organs at the flowering stage (Vergun et al., 2019). *Thymus* species extracts demonstrated reducing power in the range of 87.56–160.94 mg TE.g^{-1} (Vergun et al., 2022).

Polyphenol compounds demonstrated the scavenging ability of free radicals which determines the antioxidant activity of raw materials (Tosun et al., 2009). As a result, the studied parameters of both *S. officinalis* and *S. sclarea* extracts showed a very strong correlation between all investigated parameters and the molybdenum-reducing power of extracts (Table 1). So, a very strong correlation was found between phenolic acid content and reducing power of extracts (r = 0.927), total phenolic content and reducing power of extract (r = 0.810), and total flavonoid content and molybdenum reducing power of extract (r = 0.759) for *S. officinalis*. A strong correlation was also found between both methods of antioxidant activity determination (r = 0.732). A very strong relations determined between free radical scavenging activity and phenolic acid content (r = 0.868), flavonoid content (r = 0.732), and phenolic content (r = 0.723).

It should be noted that a weak correlation was found between free radical scavenging activity and all groups of phenolic compounds of *S. sclarea* extracts. However, molybdenum reducing power of extracts strong correlated with total polyphenol content (r = 0.909), total phenolic acids (r = 0.887), and total flavonoid content (r = 0.802).

The study of antioxidant activity and polyphenol content of different herbs showed that the correlation between investigated parameters depended on species and extracts (Kiselova et al., 2006). Comparing with other species showed that the reducing power of extracts had a very strong relationship with polyphenols,

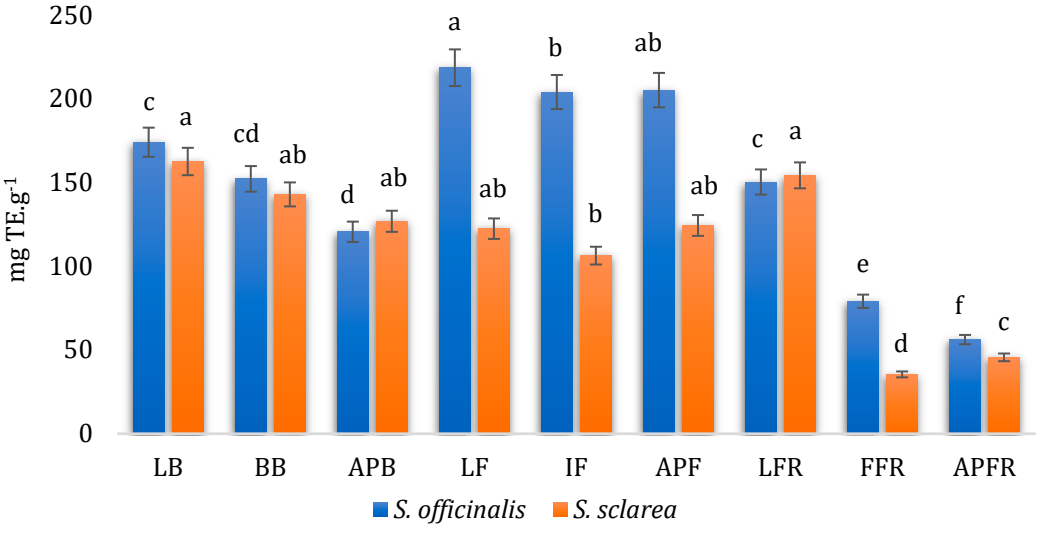

Figure 6 The molybdenum-reducing power of ethanol extracts of *Salvia officinalis* L. and *S. sclarea* L.
TE – Trolox equivalent; LB – leaves at the budding; BB – buds at the budding; APB – above-ground part of the plant at the budding stage; LF – leaves at the flowering; IF – inflorescences at the flowering stage; APF – above-ground part of the plant at the flowering stage; LFR – leaves at the fruitage; FFR – fruits at the fruitage; APFR – above-ground part of the plant at the fruitage. Different superscripts in each column indicate the significant differences in the mean at p <0.05

Table 1 Correlation analysis between antioxidant parameters of *Salvia* spp.

Parameter	TPC	TPAC	TFC	RSA	MRP
			S. officinalis		
TPAC	0.800**	1	0.776**	0.868**	0.927**
TFC	0.928*	0.776**	1	0.732**	0.759**
RSA	0.723**	0.868**	0.732**	1	0.732**
MRP	0.810**	0.927**	0.759**	0.732**	1
			S. sclarea		
TPAC	0.882**	1	0.643	-0.036	0.887**
TFC	0.840**	0.643*	1	0.266	0.802**
RSA	0.284*	-0.036	0.266*	1	-0.042
MRP	0.909**	0.887**	0.802**	-0.042	1

Note: TPC – total phenolic compounds; TPAC – total phenolic acid content; TFC – total flavonoid content; RSA – free radical scavenging activity; MRP – molybdenum reducing the power of extracts. ** Correlation is significant at p ≤0.01; * correlation is significant at p ≤0.05

flavonoids, and phenolic acids (r = 0.906–0.980), however, between both methods of antioxidant activity determination found a weak or negative correlation in case of *Scutellaria baicalensis* extracts (Vergun et al., 2019). However, the investigation of extracts of certain Lamiaceae species showed that the ratio between polyphenol compounds and method of antioxidant activity can be varied and differ from other results. The study of *Thymus* species demonstrated that values of the coefficient of correlation depended on species, so, *Th. vulgaris* extracts characterized by the strong correlation between polyphenol compounds and two assays of detection of antioxidant activity compared with other species (Vergun et al., 2022).

Conclusions

Thus, the plant raw material of two investigated *Salvia* species is a source of polyphenol compounds with high antioxidant activity. The distribution of polyphenols, phenolic acids, and flavonoids was uneven and depended on species, stage of growth, and part of the plant. The highest values of investigated parameters were found in leaf and inflorescences extracts. The minimal values of polyphenol compound content, flavonoid content, and free radical scavenging activity of extracts were found for *S. officinalis*, whereas phenolic acid content and molybdenum-reducing power of extracts for *S. sclarea*. The maximal values of phenolic acid content and molybdenum-reducing power of extracts were determined for *S. officinalis*, whereas total phenolic compounds content, flavonoid content, and free radical scavenging of extracts for *S. sclarea*. Both *S. officinalis* and *S. sclarea* extracts demonstrated a strong correlation between molybdenum-reducing power and all investigated polyphenol group compounds. Free radical scavenging activity of *S. sclarea* extracts demonstrated a weak correlation with investigated compounds. Thus, this study showed that *S. officinalis* and *S. sclarea* extracts are a source of antioxidant compounds that can be used in the pharmaceutical and food industries.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

Funding

This work was supported by grant Visegrad Fund and SAIA (Slovakian National Scholarship Program) for the scholarship for the research during which the presented knowledge was obtained.

Acknowledgements

The publication was prepared with the active participation of researchers in International Network AgroBio*Net*.

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

OPEN ACCESS

Priestia endophytica bacteria stimulate *Rhodiola rosea* L. *in vitro* growth

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Article Details:
Received:2022-07-06
Accepted:2022-08-03
Available online:2022-11-30

DOI: https://doi.org/10.15414/ainhlq.2022.0016

Soil microorganisms, in particular so-called plant growth promoting rhizobacteria (PGPR), can positively affect plants, stimulating growth by changing their metabolism. In addition, these bacteria synthesize hormone-like chemicals that can influence the formation of primary roots. This work was aimed to determine the possibility of using the sterile cell-free cultural medium obtained after the growth of endophytic bacteria *Priestia endophytica* UKM B-7515 strain (test solution) to stimulate the rooting of a valuable medicinal plant *Rhodiola rosea* L. (golden root) shoots in *in vitro* conditions and to study the physiological characteristics of the response of plants to treatment with such a medium. Single treatment of golden root shoots with a sterile cultural fluid has led to stimulation of the growth of the root system. The stimulating effect was manifested already at the beginning of cultivation. In particular, in seven days, the average number of formed roots on one shoot was 3.6 ±0.7 in the control and 7.5 ±2.1 in the experimental variant. After 28 days, the rooting of shoots that were treated with the test solution occurred more intensively. Thus, the number of roots formed on one shoot in the control and experimental variants was 7.9 ±1.2 and 16.2 ±4.8, respectively. Treated plants exceeded the control one in parameters of root weight. In particular, the weight of the roots in the control was 10.1 ±4.0 and in the experimental plants – 18.6 ±6.3. Thus, the culture medium obtained after the cultivation of *P. endophytica* UKM B-7515 bacteria without the microorganisms themselves was able to stimulate the process of rooting. Therefore, such a solution can be used for rooting plant shoots that need to be propagated in *in vitro* culture.

Keywords: *Rhodiola rosea*, *Priestia endophytica*, plant growth stimulation, indole-3-acetic acids, plant-bacteria interaction

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Introduction

The development of methods of cultivation of rare plants with a rather small area is of considerable interest especially if such plants can synthesize a number of valuable biologically active compounds. *Rhodiola* spp. belongs to such plants. The plants grow in the arctic regions of Europe and Asia. Many *Rhodiola* species, including *Rhodiola rosea* L. (golden root), demonstrated numerous bioactivities. In particular, the plants possess antidepressant, anti-inflammatory, adaptogenic, and antitumor properties (Chiang et al., 2015). The cultivation of golden root plants under sterile conditions is a necessary element of the biotechnology of these plants. In particular, such plants can be used to study the peculiarities of the synthesis of biologically active compounds that are characteristic of *R. rosea*. In addition, *in vitro* grown plants are necessary for the development of technologies for the genetic transformation of these plants.

The growing demand for *R. rosea* plants requires the availability of plant material. However, wild harvest cannot meet this demand. That is why methods of microclonal propagation of these plants in *in vitro* culture have been developed. Usually, various plant growth regulators are used to root the shoots formed in sterile conditions. In particular, indole-3-butyric and indolyl-3-acetic acids were used for the stimulation of *R. rosea* shoots rooting *in vitro* (Tasheva and Kosturkova, 2010). At the same time, this process can probably be stimulated by using soil microorganisms, in particular, the so-called plant growth-promoting rhizobacteria (PGPR). This is possible due to the production of plant hormone-like chemicals by these bacteria.

The study of the interaction of microorganisms and plants is one of the important aspects of evaluation ways and mechanisms of adaptation. This is primarily because microorganisms are part of the biocenosis of the soil on which plants grow. Plants synthesize and excrete compounds that are a source of nutrition for microorganisms. At the same time, the latter, in turn, are able to use the compounds contained in the soil, making them bioavailable to plants and thus promoting better plant growth.

Soil microorganisms can positively affect plants, stimulating their growth by changing their metabolism. In particular, it is known that bacteria of the *Bacillus* genus, which are the component of soil microbiocenosis, are able not only to increase the bioavailability of chemical elements for plants (Kang et al., 2014; Kang et al., 2015a; Yousuf et al., 2017) but also increase the resistance of plants to stress factors and pathogenic microflora (Gururani et al., 2013; Bashir et al., 2021) and synthesize growth-stimulating compounds (Kang et al., 2015b). Such features of plant growth-promoting bacteria are the basis for the development of technologies for the use of these microorganisms to stimulate plant growth, protect them from pathogens and increase resistance to stress factors (Sharma et al., 2022).

It should be noted the perspective of PGPR using to stimulate plant growth *in vitro*. Obviously, the bacteria cannot be used in the case of plant cultivation in sterile conditions. At the same time, this approach is possible due to the fact that culture fluid obtained during the cultivation of the bacteria contains compounds that can affect plant growth (Shao et al., 2015). Thanks to the presence of such compounds as indole-3-acetic acid, the resulting culture fluid can be used for rooting plant shoots *in vitro* and stimulating the growth of the root system. Therefore, this approach is of special interest for the cultivation of valuable medicinal plants in sterile conditions.

Study of the possibility of using free of cells culture medium obtained after the growth of *Priestia endophytica* UKM B-7515 bacteria to stimulate the growth of *R. rosea* in *in vitro* conditions was the purpose of this work. Some biosynthetic parameters of seedlings were also determined (the content of photosynthetic pigments; total content of flavonoids; antioxidant activity).

Material and methodology

Bacterial test solution preparation and plant cultivation

Priestia endophytica UKM B-7515 strain from the Ukrainian collection of microorganisms of the D.K. Zabolotny Institute of Microbiology and Virology NAS of Ukraine was used in the study. The bacteria were cultivated in liquid LB medium at 37 °C for 24 h with periodic stirring (180 rpm). The culture fluid was separated from the cell biomass by centrifugation at 9000 rpm (Eppendorf Centrifuge 5415C) for 10 minutes. The supernatant was sterilized by filtration through a filter with a pore diameter of 0.2 µm (Sartorius, Minisart) and diluted with sterile distilled water up to the concentration of 20% to obtain the test solution.

Biological material

Rhodiola rosea plants from the *in vitro* collection of the Laboratory of Adaptational Biotechnology, Institute

of Cell Biology and Genetic Engineering, NAS of Ukraine, were used in the experiment. The apical parts of the shoots were separated and transferred to Petri dishes with the solidified half-strength Murashige and Skoog nutrient medium (Duchefa Biochemie, Netherlands) containing 2% sucrose. 30 µl of sterile test solution was applied to the lower part of the shoots. In 28 days of cultivation at a temperature of +24 °C the plants were removed from the medium and washed with distilled water. Morphometric and biosynthetic parameters of seedlings were determined (weight of the shoots and roots; content of photosynthetic pigments; total content of flavonoids; antioxidant activity).

Chemicals

For the plants cultivation Murashige and Skoog solid nutrient medium (Duchefa, Netherlands) was used. The reagents ($NaNO_2$, $AlCl_3$, NaOH, 2,2-diphenyl-1-picrylhydrazyl radical) were of analytical grade (Sigma-Aldrich). All solutions were prepared using deinized water.

Total flavonoid content assay

Total flavonoid content was studied by a modified method (Matvieieva et al., 2019). Before this study the plants were homogenized in 70% ethanol, the resulting extracts were centrifuged for 10 min at 10000 rpm (Eppendorf Centrifuge 5415C), and the supernatants were used for flavonoid content assay. The absorbance of the samples was measured at 510 nm using the spectrophotometer Fluorat-02 Panorama. Total flavonoid content was calculated by the calibration plot: C(rutin) = 0.7889D (R^2 = 0.9928) and expressed as milligrams per gram of plant fresh weight in rutin equivalent (mg RE.g^{-1} FW).

Antioxidant activity assay

The plant extracts obtained for the total content of flavonoids study were used for antioxidant activity analysis using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) by the method described in (Brand-Williams et al., 1995). The optical density of the mixture was determined at 515 nm on the Panorama Fluorate-2 spectrophotometer. The inhibition percentage was determined by the formula: $I = [(A_0 - A_1)/A0] \times 100$, where A_0 – absorbance of DPPH*; A_1 – absorbance of the sample in the reaction. Equivalent concentration (EC_{50}) was calculated as the corresponding weight of plant material needs to obtain the extract with a 50% DPPH* inhibition level.

Photosynthetic pigments assay

Pigments were determined spectrophotometrically after extraction with 70% ethanol. The material was triturated in a mortar and centrifuged for 10 min at 10000 rpm (Eppendorf Centrifuge 5415C). The supernatant was used in the study. The total concentration of green pigments (C_{a+b}) in the extracts was determined at a wavelength of 652 nm and calculated by the formula $C_{a+b} = 29\ D_{652}$ (mg.L^{-1}), where D_{652} is the optical density of the solution at a wavelength of 652 nm. The concentrations of chlorophyll *a* (C_a) and *b* (C_b) were determined at a wavelength of 665 and 649 nm and calculated by the formulas $C_a = 11.63\ D_{665}$ (mg.L^{-1}) and $C_b = 20.11\ D_{649} - 5.18\ D_{665}$ (mg.L^{-1}), where D_{665} and D_{649} were the optical densities of the solution at a wavelength of 665 and 649 nm, respectively. Carotenoids (C_c) concentration was determined at a wavelength of 440 nm by the formula C_c = 4,695 D_{440} – 0,268 C_{a+b} (mg.L^{-1}), where D_{440} was the optical density of the solution at a wavelength of 440 nm. The content of pigments was calculated according to their concentration in solution and the weight of the starting material in mg.g^{-1} of leaf weight.

Statistical analysis

All analyses were carried out in triplicate; growth experiments were provided 7–9 times. Values were represented as mean and standard deviation (SD). The data were analyzed for statistical significance using Student's *t*-test. P values less than 0.05 were considered significant. The linear regression method was applied and the coefficient of determination (R^2) was calculated for establishing the relationship between the values.

Results and discussion

Treatment of the shoots with culture fluid has led to rapid root formation. In particular, after seven days, the average number of formed roots on one shoot was 3.6 ±0.7 in the control and 7.5 ±2.1 in the experimental variants ($p < 0.016$) (Figure 1 a, b). After 28 days, the number of roots naturally increased in both variants. However, the rooting of shoots that were treated with the test solution occurred more intensively. Thus, the number of roots formed on one shoot in the control and experimental variants was 7.9 ±1.2 and 16.2 ±4.8, respectively ($p < 0.010$) (Figure 1 c, d).

The weight of the roots of the control plants in 28 days of cultivation was significantly ($p < 0.025$) lower than the same parameter of the roots of the treated plants and was 10.1 ±4.0 and 18.6 ±6.3, respectively (Figure 2). The shoots of the treated plants also had a greater

Figure 1 Formation of the roots on the control and treated *Rhodiola rosea* L. plants in 7 (a, b) and 28 (c, d) days after treatment with the test solution

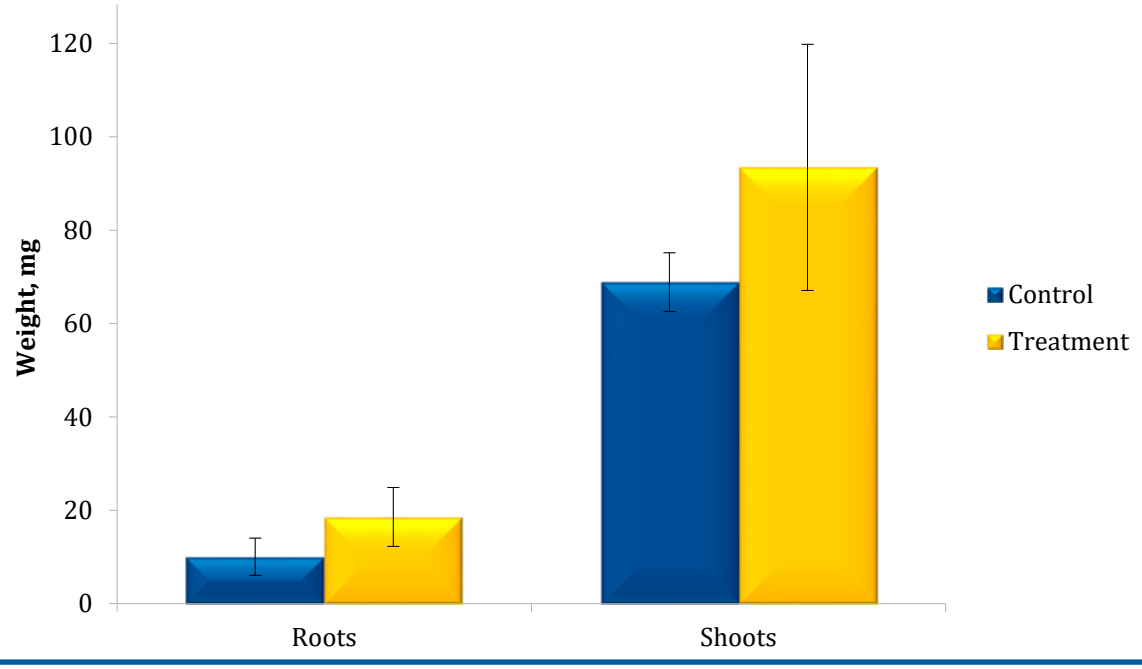

Figure 2 Weight of the roots and shoots of the control and treated *Rhodiola rosea* L. plants in 28 days of *in vitro* growth

(p <0.062) weight, which was 93.5 ±26.4, while the weight of the shoots of the control plants was equal to 68.9 ±22.1. Thus, a single treatment of *R. rosea* shoots with a cultural fluid in *in vitro* conditions has led to a significant stimulation of the growth of the root system. The stimulating effect was manifested already at the beginning of cultivation.

We used the bacterial strain isolated from cotton plants and described earlier (Reva et al., 2002). Previously, the chemical composition of the culture medium was determined after one day of cultivation of the bacteria. In particular, indol-3-butyric and indole-3-acetic acids (IAA), which were synthesized in the process of bacterial growth, were found in the nutrient medium for 59 and 757 μg.L^{-1}, respectively. These chemicals are known as the most common hormones of plants (auxins) which take part in the regulation of various plant growth processes. The compounds can be synthesized also in different microorganisms. In particular, *Pseudomonas*, *Rhizobium*, *Azospirillum*, *Enterobacter*, *Azotobacter*, *Klebsiella*, *Alcaligenes*, *Pantoea* and *Streptomyces* were found to produce IAA (Apine and Jadhav, 2011). IAA affects the induction of lateral and adventitious root formation in plants (McSteen, 2010). Park et al. (2017) demonstrated that adding IAA exhibited the highest levels of shoot and root and the fresh weight of common buckwheat plants. IAA also affected increasing the size of xylem cells (Uggla et al., 1996) and stimulated plant growth (Chhetri et al., 2022). This hormone demonstrated the properties of signaling molecules in plant-microbe interactions, taking part in the symbiosis between microorganisms and host plants (Spaepen et al., 2007; Malhotra and Srivastava, 2009; Duca et al., 1014). Based on the above data, it can be assumed that the obtained in our experiments effect of stimulating the growth of *R. rosea* plants, in particular, increasing the weight of the root system, may be associated with the presence of IAA in the test solution. Some biochemical parameters of the plant of both experimental and control variants were studied (Figure 3).

In the study of Sharma et al. (2022), treatment of plants with *Priestia endophytica* SK1 bacteria stimulated not only plant growth but also increased nitrogen and phosphorus content, as well as the concentration of phenolic compounds in fenugreek plants. In our experiments, there we no statistical differences in total flavonoid content (p <0.5) and antioxidant activity (p <0.95) in the control and treated plants. In particular, the content of flavonoids in the control plants was 1.2 0.3 mg RE.g^{-1} FW, and in treated plants – 1.0 ±0.1 mg RE.g^{-1} FW. A similar absence of significant changes was found in the analysis of the content of photosynthetic pigments and carotenoids. This lack of change can be explained by the fact that the test solution was used in a small amount (only 30 μl per shoot). Apparently, this amount was sufficient to stimulate the process of shoot formation, probably due to the presence of IAA. However, higher concentrations of active compounds are required to initiate changes in the synthesis of secondary metabolites. This assumption is confirmed, in particular, by the results of studies on the effect of

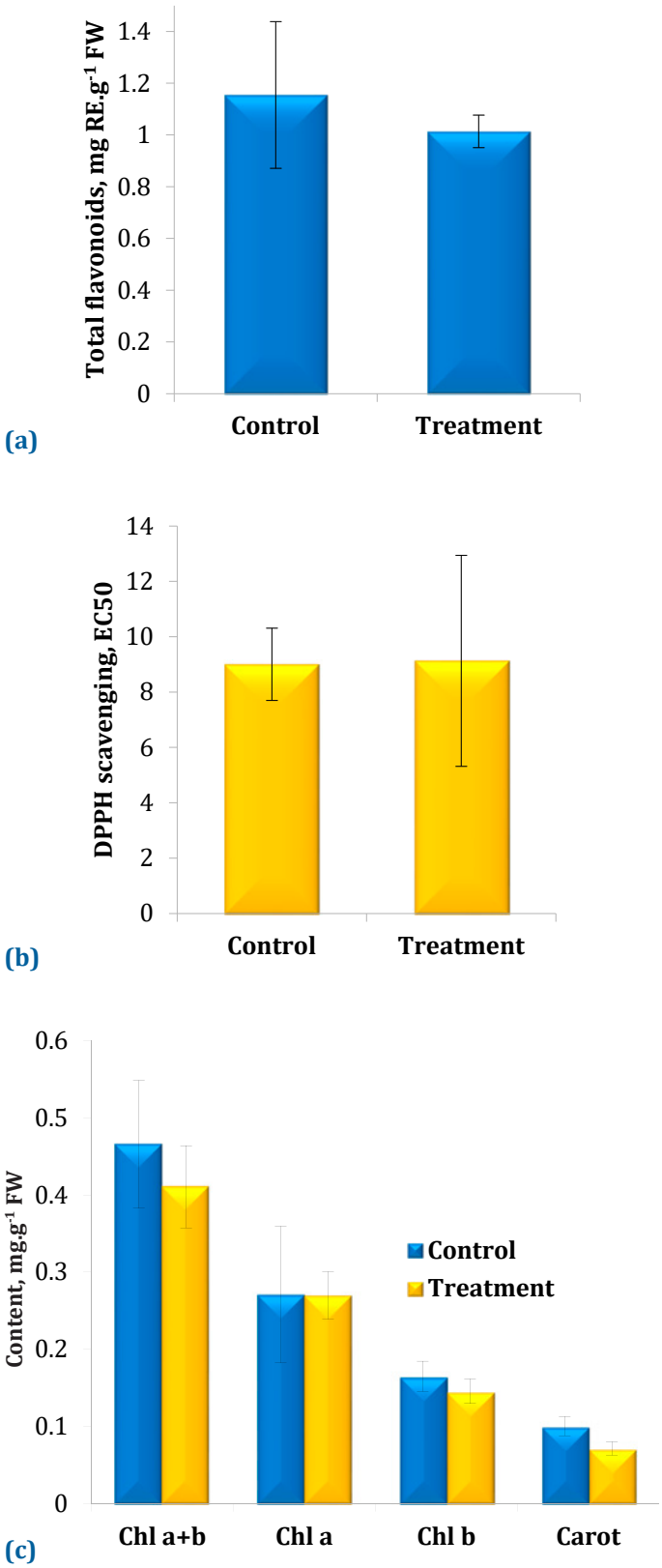

Figure 3 Total flavonoid content (a), DPPH scavenging activity (b), and photosynthetic pigments content (c) in the shoots of the control and treated *Rhodiola rosea* L. plants in 28 days of *in vitro* growth

IAA on plant growth and on the secondary metabolism of buckwheat (Park et al., 2017). Authors found that the treatment of plants with IAA at a concentration of up to 1.0 mg.L^{-1} has led not only to the stimulation of root growth, but also to an increase in the content of the total phenolic compounds and some flavonoids, in particular, 4-hydroxybenzoic acid, catechin, chlorogenic acid, caffeic acid, epicatechin, rutin, and quercetin. Treatment of tomato plants with *Bacillus licheniformis*, *Priestia megaterium*, *Bacillus subtilis*, and *Bacillus amyloliquefaciens* resulted in an increase in dry weight, the photosynthetic rate, and the content of carotenoids (Katsenios et al., 2021). However, the above studies used bacteria and not sterile culture fluid. Obviously, this can explain the lack of effect of treatment with the test solution on the content of flavonoids and photosynthetic pigments in our experiments.

Conclusions

Thus, it was established that a single treatment of *R. rosea* shoots with a sterile culture medium obtained after the cultivation of *Priestia endophytica* UKM B-7515 bacteria allows stimulating significantly the formation of roots. However, this treatment did not affect the synthesis of flavonoids, the level of antioxidant activity, and the content of photosynthetic pigments. The result indicates the possibility of using free of bacterial cells solution for quick and effective rooting of shoots of valuable medicinal plants in *in vitro* conditions.

Conflict of Interests

Authors declare any Conflict of Interests.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

Funding

This research was partially supported by Vishegrad Foundation *via* grant No 52210687.

Acknowledgements

The publication was prepared with the active participation of researchers in International Network AgroBio*Net*.

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Review

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Importance of old and local apple cultivars

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Article Details:
Received:2022-07-06
Accepted:2022-08-04
Available online:2022-11-30

DOI: https://doi.org/10.15414/ainhlq.2022.0017

Apples are among the most consumed fruits in the world. After China, the European Union is one of the biggest producers of apples. Due to the appropriate soil and climatic conditions, Poland is a leading producer of these fruits in the world and the European Union. Currently, the cultivar structure on the European market is limited to about 12 varieties. This leads to the genetic impoverishment and loss of many local cultivars, which, due to the unattractive external appearance of apples or the alternation of fruiting, are losing to the currently popular standards. The breeding of new cultivars is based on a limited number of ancestors, which poses the risk of reduced genetic diversity of the cultivars. The decline in biodiversity is also due to crop specialization. In every region of the world where apple cultivation has developed, there are many cultivars of unknown origin, which are referred to as local cultivars. They often have unique nutritional values or traits that enable them to survive. The preservation of these cultivars is justified due to the possibility of their use in breeding new cultivars, including those resistant to diseases. They can also be used in the food industry for the production of juices, cider, and high-percentage distillates, as well as a functional food. In addition, due to the higher content of some health-promoting ingredients, they are suitable for the production of, for example, anti-aging cosmetics. There are few native old cultivars in Poland. Before World War II, apple trees of English, French, German, Italian, Dutch, Belgian, Czech, Russian, and American origin predominated in Polish orchards. Old and local cultivars have remained only in home orchards, but due to the relatively short life of apple trees, they are in danger of becoming extinct. Poland undertook the protection of old and local cultivars by ratifying the Convention on Biological Diversity of Rio de Janeiro in 1992. The collection and preservation of these apple cultivars are carried out by research centres and Botanical Gardens, among others in Warsaw, Poznań, Bolestraszyce, Drawa, and Lublin.

Keywords: *Malus domestica*, biodiversity, apple domestication

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Introduction

The volume of apple production in the world and Poland

Apples are among the most consumed fruits in the world. After China, the European Union (EU) is one of the biggest producers of apples. Inside the EU, the biggest producers are Poland, Italy, France, and Germany (Jakobek et al., 2020). The dominant cultivars are Golden Delicious (21%), Gala (11%), and Idared (9%). Due to the appropriate soil and climatic conditions, Poland is a leading producer of these fruits in the world and the European Union. Apple is the national fruit of Poles due to its production volume and availability throughout the year. It is one of the most eaten fresh fruit and is a valuable raw material for processing. Apple yields in Poland amount to 83% of all fruit crops, and the area dedicated to apple cultivation is 62% of the area of all fruit plants. This makes apple cultivation monoculture, attracting the accumulation of harmful pests, especially those with a narrow range of hosts such as *Laspeyresia pomonella* (codling moths), *Aphis pomi* (apple aphid) or *Venturia inaequalis* fungi – the culprits of apple scab and *Podosphaera leucotricha* causing apple mildew. This is also intensified by the introduction of new, nobler cultivars, which often lack the defense mechanisms of their wild ancestors. The cultivar structure in the European market is limited to about 12 varieties (Hecke, 2006). Donno et al. (2012) stated that in Italy, 'Golden Delicious' is dominant in over 70% of the orchards. This leads to genetic impoverishment and the loss of many local cultivars, which, due to the unattractive external appearance of apples or the alternation of fruiting, lose to the currently popular standards (Jemrić et al., 2013).

The history of apple domestication

All apple cultivars belong to the conventional apple tree species (*Malus* × *domestica* Borkh.), which does not occur in its natural state. It was created to systematize the cultivars developed over the centuries. Initially, the species found in the Caucasus was considered to be the ancestor of apple trees: *M. pumila* Mill. (Figure 1). and common throughout Europe *M. sylvestris* Mill. It is currently believed that many apple cultivars arose from the hybridization of the mentioned species with *M. sieversii* (Ledeb.) M.Roem., *M. orientalis* Uglitzk. and *M. baccata* (L.) Borkh.

Plants from the rosacea family (Rosacae), and the pome subfamily (Pomoideae), to which the apple tree (*Malus*) belongs, did not differentiate for 50 million years of their existence (DeVore and Pigg, 2007). The

Figure 1 Apple fruits of *Malus pumila* Mill.

first differentiation took place only in the Oligocene (Xiang et al., 2016), and large-fruited forms *Malus*, *Pyrus*, and *Cydonia* evolved only in the late Miocene, i.e. 11.6–5.3 million years ago. Climate changes from the late Miocene to the Pleistocene caused fluctuations in the range of many plant species. The trees were not allowed to cross the glacier barriers during the peak ice age. The northern temperate apple tree has a very fragmented population, limited to its place of occurrence, as many of its seed distributors have ceased to exist as a result of glaciation (Pollegioni et al., 2017). Spreading of genetic form of apple trees is more effective by dispersing the seeds than pollen. Pollen may be carried up to a maximum of 10.7 km from the mother plant (Reim et al., 2015). Thus, the spread of apple trees was favoured by the development of fruit-eating megafauna: bears, deer, and humans. A breakthrough stage in the formation of the present apple tree cultivars was the beginning of the migrationrelated to the Silk Road in the late Holocene. Migrant people began to carry apples or apple seedlings, which caused different apple populations to spread across two continents. Hybridization of species was made possible by pollinating insects, and humans, through the selection of valuable plants and their further vegetative reproduction, contributed to the emergence of *M.* × *domestica*. Thus, the emergence of over 10 thousand existing apple cultivars took about 3,000 years (Splenger, 2019).

The primary method of apple domestication was by moving plants with valuable traits to the neighborhood of homes. Over time, humans have learned to reproduce

plants vegetatively through grafting or budding. They described the techniques of grafting in their tracts: Hippocrates 424 BC (Mudge et al., 2009), Theophrastus (371–287 BC), Katon (234–149 BC) and Pliny the Elder (234–149 BC) (Juniper and Mabberley, 2006). The Greeks and Romans spread the "*domestica*ted apples" across the European continent (Coart et al., 2006). The simplest breeding method was the selection of seedlings obtained by natural pollination. Then, man learned to cross-breed or search for mutations. Some of the cultivars were grown locally, while others were distributed over larger geographic areas in Europe (Hartmann, 2015).

The greatest diversity of apple cultivars occurred in Europe in the 19th century, where, with intensive cultivation, various cultivars were cultivated in many small orchards. At that time, over 2500 cultivars were known in England, and in the Soviet Union countries, there were 6000. Also on the American continent, starting from the 18th century, the breeding of apple cultivars began on a large scale (Dziubiak, 2006). Before Europeans conquered America, this continent was inhabited by wild forms of apple trees. The first apple trees from Europe are believed to have been planted in Jamestown, Virginia in 1607, and others – in 1629 – in the Massachusetts Bay Colony. From the first half of the 17th century, the production of apples increased and it was immediately accompanied by the production of cider. One of the American folk heroes, Johnny Appleseed, traveling around America in the years 1792–1842, spread apple seeds, thereby increasing the biodiversity of this species, some of which were characterized by prominent productivity. However, the selection of these cultivars was focused on the production of cider, so the taste and appearance of the apples were of secondary importance (Juniper and Mabberley, 2006; Goncharovska, 2021, 2022). In the years between 1804 and 1904 in the United States, more than 7,000 apple cultivar names were recorded, although some of them may be synonyms. Gayle and Henk (2016) report that 56% of the old cultivars found in the United States (before 1830) come from wild seedlings there. The other varieties come from England (27%), France (7%), Germany (3%), and Russia (3%).

Classification of apple cultivars

People differ in their preferences regarding the taste and appearance of apples (Bonany et al., 2014), but nowadays taste and crumbly flesh are the basic features that prove their high quality (Yue and Trong, 2011). Intensive breeding work over the centuries contributed to the creation of over 10 thousand cultivars. Pomologists tried to systematize them. The different cultivars can be distinguished by their typical external and internal characteristics. The Diel-Lucas classification, popular at the turn of the 19th and 20th centuries, was based on the morphological features of apples and distinguished 15 classes (Lucas and Medicus, 1878). The aforementioned classification was based, for example, on the shape of apples, so in the Calvia class there were cultivars with a characteristic narrow ribbing around the calyx, renets had a dry russet skin and coarse-grained flesh with a wine taste, and the Rambury's were large, flattened and asymmetrical. With time, many new cultivars were created, especially the American ones, for which the Diel-Lucas classification was outdated. Also, the development of nursery techniques and the introduction of dwarf rootstocks have led to major changes in the morphology and physiology of the cultivars improved on them. It is also influenced by the climatic and soil conditions as well as the cultivation treatments used in orchards. Moreover, it is difficult to introduce specific systematics here, because all cultivars constitute the same botanical species. Currently, pomologists classify cultivars according to the date of ripening and shelf life (Rejman, 1994).

In the last 20 years, molecular tools to characterize the diversity of apple cultivars have been implemented (Guilford et al., 1997; Hokanson et al., 1998). Such methods consist of direct analysis of DNA that can be isolated from the plant tissue independently of the phenological stage of the tree and is not influenced by the environment. The most commonly used technique is the analysis of simple sequence repeats (SSRs) or microsatellite markers, which due to their repeatability have been used to describe the genetic resources of apples in many countries (Patzak et al., 2012; Baric et al., 2020). Ordrige et al. (2018), used the Diversity array technology (DarT) markers of genetic diversity to compare 2,000 cultivars. Using the Jaccard similarity coefficient, they proposed several new varietal origins than were previously documented. For example, for the cultivar James Grieve, whose origin was described as a free pollination seedling from 'Potts seedling', they indicated that it was a cross between 'Potts seedling' and 'Cox's Orange Pippin'. Migicovsky (2016) used the correlation of traits between individual phenotypes of cultivars, based on which they were able to characterize apples from the Old World or New World, with a basic skin colour (green/yellow), used for cider or other purposes (dessert/cooking), late (October/November) and early (August/September). For example, New World apples were generally larger than Old World

apples. Old World cultivars were less red and more russet. This proves that the breeding program of the New World cultivars was aimed at obtaining cultivars with larger, better-colored fruit, and less sensitivity to russeting. They also found that the fruits of the cider cultivars were smaller and their flesh oxidized faster than that of dessert/kitchen apples. Although many breeding programs have been developed since the 1920s to obtain new apple cultivars, still very important in the structure of the current orchards, cultivars such as 'Golden Delicious', Red Delicious, and 'Granny Smith' were created as seedlings. It is only in recent decades that the cultivation of new cultivars, obtained through deliberate breeding, has become more important. However, this breeding is based on a limited number of ancestors, which creates the risk of reduced genetic diversity in cultivars (Noiton and Shelbourne, 1992). Based on the available literature, Noiton and Alspach (1996) analyzed how 439 new cultivars were created, i.e., those that appeared in production since the 1970s. They found that the creation of 64% of these varieties is based on 5 clones: 'McIntosh', Golden Delicious', 'Jonathan', 'Cox's Orange Pippin' and 'Red Delicious'. The use of only these five valuable cultivars may be due to a lack of information about other valuable germplasm cultures and a reluctance to test unknown parents.

Validity of preserving old cultivars

The importance of biodiversity. As a result of crop specialization, there has been a decline in biodiversity, which also applies to apple trees (Fowler and Mooney, 1990). There is a decline in the number of commonly cultivated varieties around the world. Miller (2014) reports that out of 15,000–16,000 apple cultivars in North America, now only 3 thousand are available in horticulture, but also over 80% of these cultivars are threatened with extinction. Only a few nurseries offer the sale of these cultivars. Nowadays, many experimental programs promote the conservation of biodiversity. Biodiversity is the diversification of life at all levels of an organization. Since 1992, the protection of the natural environment and biodiversity has been an integral part of the Common Agricultural Policy. Biodiversity is fundamental to the evolution and durability of life support systems in the biosphere. The higher the biological diversity of a given ecosystem, the more resistant it is to various natural disasters (droughts, floods, diseases, hailstorms, etc.) and artificial ones (chemical and radioactive contamination, the introduction of alien species, noise, global warming, etc.). To protect biodiversity, it is necessary to anticipate, prevent and combat the causes of its decline or disappearance. The importance of biodiversity protection results primarily from the need to maintain a balance in nature.

Old cultivars are gene pools the use of which can be of great economic importance. Promoting the preservation of old cultivars, as a rule, attention is paid to the protection of genetic resources and saving vanishing genotypes.

- Local cultivars of crops: increase the species and varietal diversity of crops, which prevents the simplification of crop rotation and ensures the diversity of habitats,
- In general, they have lower cultivation requirements, what allows to limit the use of fertilization and plant protection products,
- Some of them are especially useful in extensive production systems and for keeping agricultural production in marginal lands.

Preserving plant gene resources is the only way to guarantee their availability now and in the future. It is difficult to predict changes in the environment and all human needs, so it is necessary to maintain the widest possible genetic variability of plants. The more genetically diverse the plant material, the greater the chance of finding forms with useful features in plant breeding and plant production.

In every region of the world where the cultivation of apple trees has developed, there are many varieties of unknown origin, which are referred to as local cultivars. They are often characterized by unique nutritional values or qualities that enable them to survive (Király et al., 2020). In some countries, e.g. Hungary (Király et al., 2020), Bosnia-Herzegovina (Stanivuković et al., 2017), Montenegro (Božović et al., 2013), the United States (Miller, 2014), Iran (Damyar et al., 2007), the presence of so-called local cultivars, i.e. those that can adapt to the conditions prevailing in a given region, is noted. For many years, they have been grown on a small scale for local markets.

One way to preserve the old cultivars is to create embryonic plasma repositories to prevent the future narrowing of the genetic base (Way et al., 1990). The next step is to use this diverse germ plasma in breeding programs rather than relying only on inbreeding (Noiton and Shelbourne, 1992). In many countries, repositories in the form of orchards are established based on the accumulated old and local cultivars. It is necessary to identify and evaluate genotypes based on the morphometrical and biochemical traits in various conditions, as evidenced by the many authors (Ivanišová et al., 2017; Grygorieva et al., 2017a,b,

2018a,b; Fatrcová Šramková et al., 2019; Vergun et al., 2020; Horčinová Sedláčková et al., 2020, 2021). In England, there is an increased interest in setting up a lot of small community orchards with 100-old trees. The Newquay community orchard in Cornwall was founded in 2015 with a £66,000 crowdfunding appeal. More than 2,000 trees, including 120 local heritage cultivars, have been planted on land donated by the Duchy of Cornwall. In Lithuania, an attempt was made to establish a modern orchard based on the oldest cultivar there: Ničnera zemeņu (Edrbeerapfel Nitschners) and Trebū sēklaudzis and Mālābele, on a semi-dwarf rootstock MM 106. Experience shows that the tested varieties require refinement of harvesting technology and care work. The cv. Mālābele, as a late summer variety, needs to be harvested several times. The cv. Trebū sēklaudzis, due to its strong growth, requires a wider spacing in the row (1.5–2.0 m) and the formation of crowns in slender spindle, as well as thinning of buds, to improve the quality of the fruit (Rubauskis and Borisova, 2021).

Nutritional and health-promoting characteristics of old and local cultivars

Apple, as a fruit, provides man with many valuable chemicals such as carbohydrates, vitamins, mineral compounds and fibres, pectin and various polyphenols, while it is characterized by a low concentration of protein and fat.

Currently, in many countries, studies are being carried out on the quality characteristics of the fruits of old and local cultivars. Unfortunately, determining what the old and new cultivar is not unambiguous. 'Golden Delicious' in some experiments is considered an old cultivar (Wojdyło et al., 2008; Mitre et al., 2009), and in some as a representative of new cultivars (Kschonsek et al., 2018; Morresi et al., 2018; Király et al., 2020; Ceci et al., 2021). This is probably due to the fact that although this cultivar was bred in the nineteenth century, its share in the varietal structure of many countries is still significant.

Oszmiański et al. (2018), while determining the chemical composition of 22 old cultivars, noted that the dry matter content was from 1.30 to 17.12 g.100 g^{-1}, soluble solids content from 10.50 to 14.70 Bx, fructose from 3.96 to 8.52 g.100 g^{-1} f.w. glucose from 0.94 to 2.96 g.100 g^{-1} f.w., sucrose from 0.06 to 2.72 g.100 g^{-1} f.w., total sugar from 7.41 to 11.99 g.100 g^{-1} f.w., total acidity from 0.17 to 1.07 g.100 g^{-1}, pectin content from 0.65 to 2.24 g.100 g^{-1}. The concentration of polyphenols in 22 apple cultivars ranged from 1,348.40 to 4,310.52 mg.100 g^{-1} d.m. in the fruits of the Altländer Pfannkuchenapfel and Roter Trier Weinapfel cultivars; triterpenoids ranged from 466.30 to 3,753.60 μg.g^{-1} d.m. in the fruits of the Gelber Richard and Wintergoldparmane cultivars. The highest ABTS and FRAP test values were observed for the cv. Wintergoldparmane (124.71 and 80.15 μmol Trolox.g^{-1} dm) and Horneburger Pfannkuchenapfel (117.93 and 78.63 μmol Trolox.g^{-1} dm).

Wojdyło et al. (2008) draw attention to the content of health-promoting ingredients such as polyphenols, including anthocyanins, flavanols, phenolic acids, flavonols, vitamin C content, etc. Analysis of the phenolic profile in apples indicates that the main groups are procyanidins, flavan-3-ols, and chlorogenic acid, while anthocyanins and ploridzin are the smallest groups. The antioxidant activity of apples depends on the content of polyphenols, especially on the content of procyanidins/flavan-3-ols (Wojdyło et al., 2008). In the study Kschonsek et al. (2018), the pulp of new cultivars such as Elstar or Jonagold was characterized by a lower content of major phenolic groups and vitamin C compared to the pulp of old cultivars such as Ontario, Oldenburger, Goldparmäne, Berlepsch. They showed that the antioxidant capacity depends on the content of flavonols in the peel, not vitamin C. In contrast, in the pulp, the content of vitamin C determines the antioxidant properties of apples. Therefore, it is worth consuming fruits with peel. Wojdyło et al. (2008), comparing the content of polyphenols among old and new cultivars, found that new cultivars such as Ozark Gold, Julyred and Jester, were characterized by the same or higher content of bioactive ingredients compared to the old cultivar: Golden Delicious, Idared, and Jonagold.

Božović et al. (2013), assessing the apples of old cultivars growing in Montenegro, found that they ripened from mid-July to mid-October. Extremely large fruits were characterized by 'Ilinjača'' (167.50 g), 'Dunjka' (170.15 g) and 'Moračka Krstovača krupna' (182.34 g). It is noted that early fruiting cultivars such as 'Šarena petrovača' and 'Ilinjača' and late-ripening cultivars such as Aleksandrija, Limunjača and Rebrača can be used for kitchen use. In addition, cultivars with a high soluble solids content: Aleksandrija (16%), Rebrača (15.5%), Jolovača (14.6%) and Dunjka (14.5%) can be used as dessert fruits, for direct consumption.

Jakobek and Barron (2016) found that the polyphene profile of several old Croatian cultivars was similar to the American cultivars (Cortland and Russet). Some of the old autumn cultivars like 'Zimnjara' can be a good source of polyphenols due to the particularly high content of total polyphenols and dihydrochalcone

content. The cv. Adamova zvijezda was characterized by the highest values of quercetin derivative and flavanol content in the skin. They observed that parenchymal cultivar with a high proportion of phenolic acid is characterized by a low content of flavanol and vice versa.

Passafiume et al. (2021) compared the quality of fruits of old and new cultivars bred in Italy and grown in the mountainous conditions of Sicily. They found that both old and new clones are characterized by high fruit quality. The new clones are more attractive in the dessert fruit market, where the consumer is looking for apples that are better coloured and sweeter. However, the old genotypes had more vitamins: thiamine, B_5, B_2, E and C, so they can satisfy not only the niche market.

Donno et al. (2012) compared 9 local ancient cultivars: Bella di Barge, Buras, Contessa, Dominici, Gamba Fina, Grigia di Torrian, Losa, Magnana, and Runsè with the control cv. Golden Delicious found that they were characterized by a higher content of vitamin C, total polyphenols and antioxidant activity.

Morresi et al. (2018) report that old cultivars of apple trees from the Marche region of Italy are characterized by high variability. They proved that a 150 g apple can provide, depending on the variety, from 12.5 mg (cv. Gelata) to more than 500 mg (cv. Calville White Winter) of total polyphenols.

Ceci et al. (2021), comparing the chemical composition of old cultivars, commonly grown (commercial), noted that the old cultivars were distinguished by the content of polyphenolic compounds and higher antioxidant capacity.

Sometimes apples can cause allergies, but it has been found that old cultivars are better tolerated by people who have developed intolerance compared to new cultivars (Kschonsek et al., 2019).

Apples of old cultivars contain higher amounts of dihydrochalcones than commercial cultivars. These compounds have the potential to lower blood glucose levels, which may hel prevent diabetes (Kobori et al., 2012; Mei et al., 2016).

Possibilities of using old cultivars of apple trees

Breeding cultivars resistant to diseases and pests. Perennial cultivation of apple trees in large areas caused the accumulation of diseases and pests. The most dangerous diseases include apple scab (*Venturia inaequalis* Cke.) and powdery mildew of apple (*Podosphaera leucotricha* Salm.), and pests: aphids, especially aphis pomi (*Aphis pomi*) and codling moth (*Cydia pomonella*). Until now, most resistance breeding has been based on the *Malus floribunda* type 821 × Rome Beauty cross, followed by reverse crosses with noble varieties to give the new variety valuable quality characteristics (Crosby et al., 1992).

The presence of specific phenolic compounds can cause low susceptibility of apple fruits to the most important diseases. One such compound is phloridzin (a derivative of chalcone) which is a characteristic apple polyphenol. It is a phytolaexin that provides resistance to pathogens – *Venturia inaequalis* and *Erwinia amylovora* (Petkovsek et al., 2010). Other researchers (McClure et al., 2019) emphasize that flavan-3-ols are responsible for increasing resistance to *Venturia inaequalis*.

Old cultivars, due to their resistance to diseases, especially scab and powdery mildew, can be used in the expansion of such breeding programs. In addition, due to the high field resistance to these diseases, they are suitable for organic cultivation. Papp et al., 2015 studying resistance to apple scab, powdery mildew and fire blight among the old cultivars accumulated in Hungary distinguished those that can be recommended for organic cultivation: 'Batul', 'Vilmos renet', 'Pónyik', 'Sikulai', 'Tordai piros lálvil' and 'Szabadkai szeresika'. The old 'Batul' cultivar from the Carpathian Basin probably contains genes responsible for inheriting resistance to fire blight FB_MR5 and Scab Vh4Vh4. These observations are consistent with Halász et al., 2011, who indicate that the cv. Sikulai, Tordai piroskálvil, Batul, Vilmos renet, Zöld sóvári, are resistant to Ervinia amylovora and apple scab. They have S2 or S3 alleles (Broothaerts and Van Nerum, 2003). Some of these cultivars have been known in the Carpathian Basin for more than 200 years, and even date back to the time of the Turkish occupation of Transylvania. Militaru et al. (2015), comparing a dozen or so attics of Romanian cultivars, distinguished the cv. Gustaw durabil as resistant to apple scab, cv. Botane – resistant to apple mildew. In addition, the cv. Gustaw durabil and Pătul were not attacked at all by the aphis pomi, while cv. Verzi de Rădăşeni, Domneşti, Roşii de Geoagiu, Călugăreşti – by the codling moth.

Raw material in the food industry

The current consumer is looking for food produced and processed sustainably, making it safe, fresh and natural (Putnik et al., 2018). Many consumers appreciate the unique taste of old cultivars and are aware of their nutritional and health-promoting values. Thanks to this, old cultivars can be restored to the dessert fruit market. Donno et al. (2012) created a sensory profile of

ancient cultivars and distinguished several interesting features based on a sensory analysis. They found that old cultivars such as Buras, Contesse, Grigia di Torriana and Runsè show good organoleptic quality, so they could expand the local apple market. Oszmiański et al. (2018) found that some old apple fruit cultivars, especially Roter Trier Weinapfel, Wintergoldparmane and Horneburger Pfannkuchenapfel, are characterized by the highest amount of bioactive compounds and antioxidant properties. They can therefore be chosen for their potential use in commercial cultivation for the production of fruits with a valuable health-promoting nutritional effect on human health. Therefore, old apple cultivars can be a promising source of health-promoting compounds with potential health benefits. Mitre et al. (2009), assessing the yield and quality of fruits of several old cultivars: Golden Delicious, Jonathan, Starkrimson, Wagener, Kaltherer Bőhmer in Transylvania conditions, concluded that due to the abundance of yield and high quality of fruits, they should continue to be maintained in commercial orchards. On the other hand, Jeremić et al. (2013) examined the usefulness of 9 old cultivars: Gelber Bellefleur, Carevic, Celenka, Crvena Jesenska Rebrača, Paradija, Paulaner Weinapfel, Perovnjaca, Winter Banana, and Zuccalmaggio, on the MM 106 rootstock, in Croatian conditions found that they are characterized by too little weight (80–120 g) and without special treatments such as fertilization, cutting and thinning, they do not meet market standards. Horčinová Sedláčková et al. (2020, 2021) stated that some local cultivars in Slovakia, that grow wild and represent spontaneous seedlings from free pollination have a set of economically important traits and are ready to be used as potential genetic resources for a breeding program.

Apples of old cultivars are suitable for the production of valuable juices (Jakobek and Barron, 2016). Many valuable ingredients characteristic of apples are also found after their processing in juice. Iaccarino et al. (2019) studying the chemical composition of juices from ancient cultivars found that it is similar to popular apple juices. The extract content ranged from 8.1 to 14.2 3 gL^{-1}. The predominant sugar was fructose (from 30.1–78.3 gL^{-1}). The glucose content was 5.4–20.7 gL^{-1} and sucrose from 8.5 to 63.2 gL^{-1}. The dominant acid was malic acid (on average 8.8 gL^{-1}). The ratio of sugars to acids ranged from 5.5–33.1, with the values of this coefficient of 15–16 being shown to give the desired balance of sweet taste and acidity. The juice of four cultivars: Gadeskovæble, Ingersæble, Bodil Neergaard, and Barritskov Madæble were particularly rich in polyphenols, while apple juice of the cv. Mormorsæble and Antonius were characterized by a very specific smell and taste like apricot and peach. Thus, cultivars distinguished by special taste qualities can be used for the production of single-variety juices, the so-called 'vintage', which diversify the juice menu in restaurants.

Recently, the processing industry in Poland has been dynamically developing, in the production of cider. In Poland, the intensive development of this industry took place in 2013, when the Association of Fruit Growers of the Republic of Poland began to look for ways of developing industrial apples other than apple concentrate. The refinement of the Excise Tax Act, which reduces excise duty on low-percentage alcohol, encouraged many entrepreneurs to develop apples in the form of cider. The embargo introduced by Russia on Polish apples in 2014 meant that the production of these beverages amounted to 800,000 litres in 2013, in 2014 increased to 2 million litres, and in the following year to 15 million litres (Nosecka and Bugała, 2019). The development of cider production in Poland may be affected by a change in taxes, which assumed a 10% increase in excise duty on wine, beer and vodka from January 1, 2020. According to the EU Agriculture Outlook (EC 2019), there may be an increase in cider consumption in new markets from Central and Eastern Europe. Craft ciders focused on consumers looking for innovative and unique flavours have great potential (Reiss et al., 2012). The quality of cider largely depends on the quality of the apples. It is assumed that the juice for the production of cider should contain about 15% sugars, 0.2% tannins and 0.3–0.5% acids. Therefore, in Poland, different cultivars of apples are mixed to obtain the expected chemical composition of the juice. To increase the acidity of the juice, 20% juice of wild apples is added to the basic apple raw material. You can also reach for older cultivars of apples with a sour aftertaste, with a hint of bitterness, such as: 'Berner Rose', 'Reneta Landsberska', 'Żeleźniak', 'Sztetyna Czerwona', 'Kardynalskie', 'Kalwila Czerwona', 'Kazachstanskoje Jubilinnoje'. Jemrić et al. (2013) studying the quality of old apple cultivars indicated the cv. Perovnjača characterized by the highest acidity and less than 20 SSC:TA ratio, which makes it suitable for cider production.

Apples can also be used to make fruit distillates. Fruit distillates are popular spirits because of their unique taste. The taste of fruit distillates depends primarily on the fruit (primary taste), fermentation (secondary taste), distillation (tertiary taste) and ripening (quaternary flavour). Very important is the presence of suitable volatile organic compounds that affect the overall sensory characteristics of the product. Spaho

et al. (2021) studied in Bosnia and Herzegovina the suitability of old cultivars for the production of fruit distillates found that products from the cv. Prijedorska zelenika and Masnjača had an intense fruity-sour aroma that surpassed the control cv. Golden Delicious. In contrast, distillates from apples of the cv. Žuja in terms of chemical composition and sensory quality were similar to those of the cv. Golden Delicious. The alcohol obtained from the apples of the cv. Šarenika was distinguished by its aroma, but it still needs to be specially purified during distillation. Apples of the cv. Samoniklica, due to the high content of terpenes, can be a valuable flavouring additive. However, apples of the cv. Ljepocvjetka, Bobovec and Sarija are not suitable for the production of distillates due to the lack of an aromatic contribution.

Old cultivars, due to their outstanding health-promoting properties, can also be a raw material for the production of functional food (Duralijo et al., 2021). The consumption of such foods as part of a varied diet has beneficial effects that go beyond the basic nutritional values. An example of such products is functional drinks, which are, for example, a mixture of apple juice and tea (De Souza et al., 2020). Thanks to improved physicochemical properties, increased nutritional or health-promoting properties and sensory attractiveness, they can find interest among athletes, convalescents, etc. There are attempts to create flour from apple pomace, as a gluten-free product that would replace wheat flour for people with celiac disease. Flour with has a lower protein content (1.25%) and more fibre (56%) than wheat and rice flour. The total phenol content in apple flour is 4 times higher than in wheat and 7 times higher than in rice. Cakes made of such flour, despite being harder, meet the expectations of consumers (Azari et al., 2020).

Due to the high content of polyphenols, apples can also be used in the production of anti-ageing cosmetics. Morresi et al. (2018) proved that glycoxidation is responsible for skin ageing, and the polyphenols contained in apples of old varieties effectively prevent this. Barreira et al. (2019) suggests that phenolic compounds from apples can be used in skin preparations due to several beneficial properties such as antioxidant or antimicrobial effects.

Old cultivars in Poland

In Poland, horticulture developed thanks to monks and gardeners working in castle gardens. In the Middle Ages, several noble varieties of apple trees were grown, imported mainly from Germany and France. The travelling nobility also brought interesting

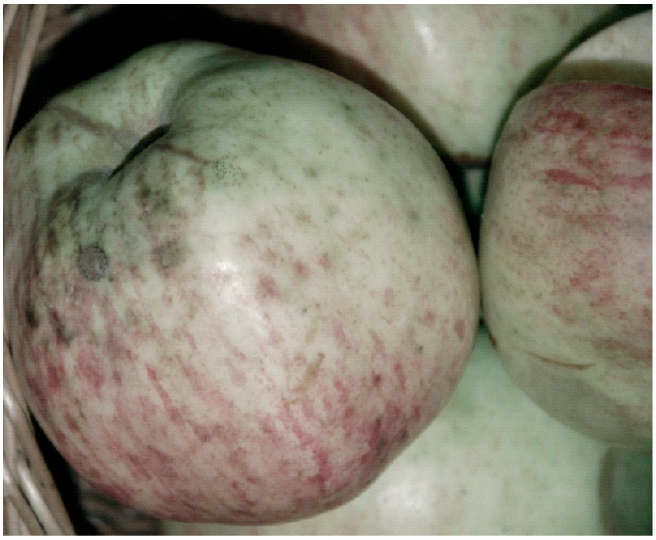

Figure 2 Cultivar Rapa Zielona

plant specimens from foreign countries. Soldiers returning from wars smuggled valuable plants. From the mid-nineteenth century, when the commodity production of apples in peasant farms began to develop, it was modelled on German and French fruit growing and apple cultivars were still imported from these countries. However, in Poland, due to the lack of schools focused on the development of horticultural sciences, purposeful breeding did not develop (Jankowski, 1923). Records of nursery catalogues from the early 30s of the twentieth century indicate Polish cultivars: Bukówka, Bursztówka Polska, Kalwaryjska, Kosztela, Ksawerówka, Papierówka Podlaska, Piękna z Rept, Profesor Jankowski, Rapa Zielona (Figure 2), Rarytas Śląski, Tyrolka Krynicka, Węgierczyk, Złota Kwidzyńska. The cv. Kosztela is associated with a legend explaining the origin of this name. Kosztela was probably bred by the Cistercians in the sixteenth century in Czerwińsk as Wierzbówka Zimowa. Planted in the palace garden of Jan III Sobieski in Wilanów, it bore fruit alternately. In the year of poor yield, Queen Marysieńka, collecting fruit from under the apple tree, assessed the crop as "kosz tylko?" (the basket only). With time, Wierzbówka adopted the name Kosztylka, and finally Kosztela (Smardzewski, 1917–1932).

The Polish people often cultivated cultivars from neighbouring countries, such as Lithuanian: Pineapple Berżenicki, Cukrówka Litewska, Malinówka Berżenicka, Reneta Litewska, Strumiłówka, Śmietankowe; Russian: Antonówka zwykła, Antonówka Półtorafuntowa, Antonówka Kamienna (Figure 3 (A)), Antonówka Kołowa (Figure 3 (B)), Charłamowska, Kandil Sinap (Figure 3 (C)); Ukrainian: Aporta; German: Kaiser Wilhelm, Grochówka (Figure 3 (D)), Żeleźniak

Figure 3 Cultivars of apples
A – Antonówka Kamienna; B – Antonówka Kołowa; C – Kandill Sinap; D – Grafsztynek Inflancki; E – Grochówka; F – Żeleźniak

(Figure 3 (E)), from the Baltic countries: Grafsztynek Inflancki (Figure 3 (F)), Oliwka Inflancka, Glogierówka (synonym Pepina Litewska).

As a result of the search for valuable cultivars before World War II, cultivars of apple trees of English, French, German, Italian, Dutch, Belgian, Czech, Russian and American origin prevailed in Polish orchards. Cultivars of apple trees imported from other countries often had names that were difficult to mention and spell, so they were changed to more familiar-sounding ones. For example, Kronselska (Figure 4a A) with the foreign-language name Pomme de Croncels or Transparente de Croncelles has settled in Poland. Other old cultivars from countries not directly bordering Poland are: Grafsztynek Prawdziwy, Fameuse (Figure 4a B), Hibernal, Glockenapfel (Figure 4a C), Golden Delicious, Granny Smith (Figure 4a D), Jonatan, Kantówka Gdańska (Figure 4b E), Kardynalskie (Figure 4b F), Koksa Pomarańczowa (Figure 4b J), Królowa Renet (Figure 4b K), Krótkonóżka Królewska (Figure 4b L), Książę Albrech Pruski, Książęca, Malinowa Oberlandzka, Reneta Landsberska (Figure 4b M), Pepina Saffron, Signe Tillisch, Piękna z Boskoop, Piękna of Barnak.

In the interwar period, there were several cultivars of apple trees in commercial production, but none came from Polish breeding. During this period, the first American cultivars were imported, e.g. Jonathan, and after the Second World War: McIntosh, Lobo, Cortland, Bankroft. The dominant varieties in orchards at that time were: Boskoop, Cesarz Wilhelm, Oliwka Żółta, Landsberska, Grochówka and summer cultivars. A clear breakthrough in the efficiency and quality of apple production occurred after 1995 when most of the trees in the orchards were replaced by cultivars on dwarf rootstocks.

Old and local cultivars have remained only in home orchards, but due to the relatively short life of apple

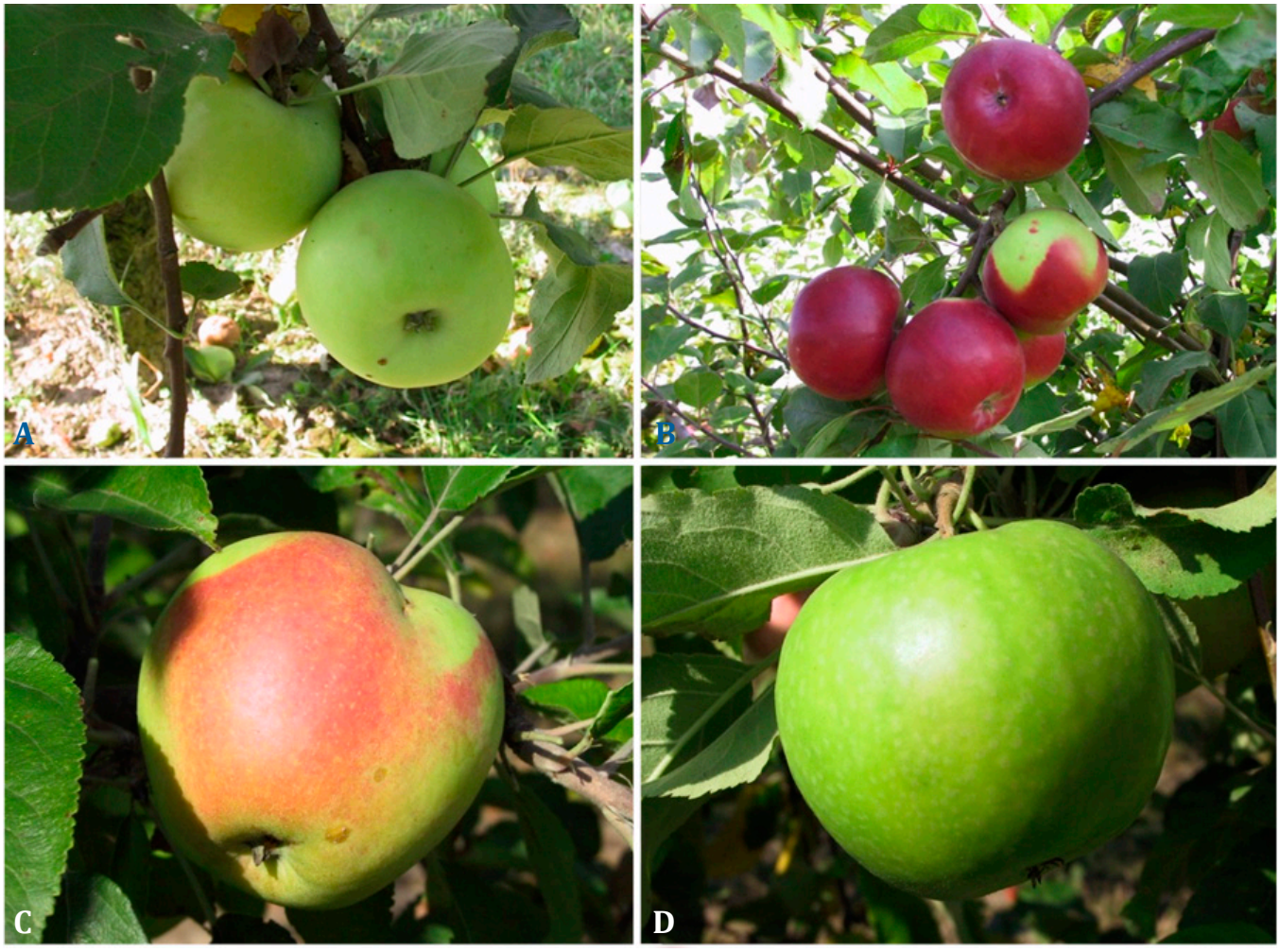

Figure 4a Cultivars of apples
A – Koronselska; B – Fameuse; C – Glockenapfel; D – Granny Smith

Figure 4b Cultivars of apples
E – Kantówka Gdańska; F – Kardynalskie; J – Koksa Pomarańczowa; K – Królowa Renet; L – Krótkonóżka Królewska; N – Reneta Landsberska

trees, they are in danger of extinction. Poland undertook to protect old and local cultivars by ratifying the Rio de Janeiro Convention on Biological Diversity in 1992. The collection and preservation of these cultivars of apple trees is carried out by research centres and Botanical Gardens m.in in Warsaw, Poznań, Bolestraszyce (Żygala et al., 2011), Drawa National Park and its neighbouring lies in a plain called Drawska Plain, which is a fragment of the lake district South Pomeranian Lake District, in the north-western part of Poland (Oszmiański et al., 2018). In Lublin, in the experimental orchard of the University of Life Sciences in Lublin, in 2021, a quarter of about 70 old and local cultivars were planted, where the material consists of trees budding on the M9 rootstock, and the "slips eye" from scions from Bolestraszyce. Also, amateur growers are increasingly interested in planting trees of old cultivars, in home orchards and organic farms, and the amended regulations allow nurserymen to produce them.

Conclusions

As a result of centuries-old cultivation and breeding of apple trees, more than 10,000 cultivars were created, including in the common, conventional species *Malus* × *domestica* Borkh. The greatest diversity in the structure of cultivated cultivars was marked in the nineteenth century, due to the intensification of the intensity of breeding work and the cultivation of apple trees in many small areas. However, around the world, with the increase in the specialization of production, the number of cultivars of apple trees decreased to about twelve. This caused a huge risk of a decline in genetic diversity. In every region of the world where apple production has developed, there are many varieties of unknown origin, which are referred to as local cultivars. They are often characterized by unique nutritional values or features that enable them to survive. The preservation of these cultivars is justified due to the possibility of their use in the breeding of new cultivars, including those resistant to diseases. They can also be used in the food industry for the production of juices, cider and high-percentage distillates, as well as functional food. In addition, due to the higher content of some health-promoting ingredients, they are suitable for the production of anti-ageing cosmetics. In Poland, old and local cultivars are preserved in collection pomological gardens, e.g. in Bolestraszyce and Lublin, and by establishing organic orchards based on these cultivars.

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

OPEN ACCESS

Antibacterial activity of ethanolic extracts of *Aglaonema commutatum* Schott and its cultivars against *Enterococcus faecalis* strains

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Article Details:
Received:2022-07-10
Accepted:2022-08-08
Available online:2022-11-30

DOI: https://doi.org/10.15414/ainhlq.2022.0018

The application of plants coming from tropical and subtropical regions in the management of bacterial infections can be considered a positive occurrence in most traditional medicine practices. Consequently, plants having antimicrobial activity against various pathogens can be considered great assets. Moreover, increased problems associated with side effects and bacterial resistance to chemical drugs have prompted us to focus on the antibacterial potentials of some plants belonging to the *Aglaonema* genus. The purpose of the current study was to examine the antibacterial activity of ethanolic extracts derived from *Aglaonema commutatum* Schott and its cultivars (Malay Beauty, Silver Queen, and Silver King) against two *Enterococcus faecalis* strains, i.e. *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ (resistant to vancomycin; sensitive to teicoplanin) and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™. These plants were cultivated under glasshouse conditions at M.M. Gryshko National Botanical Garden, National Academy of Science of Ukraine. The testing of the antibacterial activity of the plant extracts was carried out *in vitro* by the Kirby-Bauer disc diffusion technique. Results of this study revealed that the extracts derived from leaves of *A. commutatum* and cv. Silver Queen exhibited higher inhibitory activity against the growth of *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ than the extracts from cv. Melay Beauty and Silver King. Maximum *in vitro* inhibition was scored by cultivar Silver Queen, followed by *A. commutatum*, cv. Malay Beauty and Silver King. On the other hand, extracts derived from leaves of *A. commutatum* cv. Melay Beauty and Silver Queen exhibited higher inhibitory activity against the growth of *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™ than the extracts derived from the cv. Silver King and *A. commutatum*. Maximum *in vitro* inhibition was scored by cv. Malay Beauty, followed by cv. Silver Queen and Silver King, and *A. commutatum*. Therefore, this plant can be used to treat various diseases caused by *E. faecalis* strains. There is a lot of potentials for this plant to treat infections caused by these bacteria. Therefore, these plants may be helpful in the management of infections caused by *E. faecalis*, especially in traditional medicine practices. However, further research is required to understand the mechanisms involved in antimicrobial activity.

Keywords: *Aglaonema commutatum*, leaf ethanolic extracts, antibacterial activity, inhibition zones, Kirby-Bauer disc diffusion technique

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Introduction

Aglaonema, commonly called Chinese evergreens, belongs to the family Araceae (Henny et al., 2008; Li et al., 2022). The *Aglaonema* genus comprises 21 species distributed in southeast Asia, northeast India, and southern China southward through Malaysia, New Guinea, and the Philippines (Nicolson, 1969; Govaerts and Frodin, 2002; Chen et al., 2003). *Aglaonema* contains many cultivars that are important tropical foliage plants. Plants belonging to this genus readily adapt to low light and low relative humidity levels encountered under interior conditions (Henny et al., 2008; Henny and Chen, 2010). These plants have been widely cultivated by hybridization and tissue-cultured mutation selection for ornamental and medical purposes (Chen et al., 2004; Henny and Chen, 2010; Li et al., 2022).

Aglaonema plants have been widely used in recent years because of their anti-aging and longevity properties, and natural anti-allergic and anti-inflammatory properties (Kiatsongchai, 2015; Islam et al., 2019). Moreover, a decoction of the roots is drunk to treat dropsy and fever (Perry, 1980). Anti-hyperglycemic effects of N-containing sugars from *Aglaonema treubii* Engl. in diabetic mice were noted (Nojima et al., 1998). It was shown that the genus contains polyhydroxy alkaloids that exhibit glycosidase inhibitor activity (Ismail et al., 2017).

A literature survey by Roy et al. (2013) reveals that research works on antibacterial activity have been conducted on different plants of Araceae and most of the plants under investigation have shown significant activity against different pathogenic bacteria. The available data, regarding the zone of inhibitions, indicate that the bacterial strains whose activities have been inhibited most by the secondary metabolites present in the crude extracts of the plants are *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*. A maximum zone of inhibition has been observed in the case of ethanol extract obtained from the tuber of Typhonium trilobatum having a 32 mm zone of inhibition against *Staphylococcus aureus* (Roy et al., 2013).

In our previous study, we also investigated the antibacterial properties of ethanolic extracts derived from *Aglaonema commutatum* and its cultivars against various strains (Opryshko et al., 2019, 2020a,b). In the current study, we used two *Enterococcus faecalis* strains, i.e. *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ (resistant to vancomycin; sensitive to teicoplanin) and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™. *Enterococcus faecalis* is a Gram-positive pathogen that colonizes human intestinal surfaces, forming biofilms (Oh et al., 2021). It also is a common commensal organism and a prolific nosocomial pathogen that causes biofilm-associated infections and demonstrates a high resistance to many antibiotics (Willett et al., 2021). *E. faecalis* is one of the most frequently isolated bacterial species in wounds yet little is known about its pathogenic mechanisms in this setting (Chong et al., 2017). Especially, antibiotics are less effective in eradicating biofilms and better alternatives are needed (Oh et al., 2021).

In line with the growing interest in the antibacterial potential of different tropical and subtropical plants, we examined the antibacterial properties of ethanolic extracts derived from leaves of *Aglaonema commutatum* and its cultivars against two *Enterococcus faecalis* strains.

Material and methodology

Collection of plant materials and preparation of plant extracts

The leaves of *Aglaonema commutatum* Schott and its cultivars (Malay Beauty, Silver Queen, Silver King), cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanic Garden (NBG), National Academy of Sciences of Ukraine (Kyiv) in 2020. The leaves were brought into the laboratory for antimicrobial studies. Freshly sampled leaves were washed, weighed, and homogenized in 96% ethanol (in the ratio of 1 : 19, w/w) at room temperature. The extracts were then filtered and stored in glass bottles with dark walls at 4 °C. Extracts were investigated for their antimicrobial activity in 2020–2021.

Determination of the antibacterial activity of plant extracts by the disk diffusion method

The testing of the antibacterial activity of the plant extracts was carried out *in vitro* by the Kirby-Bauer disc diffusion technique (Bauer et al., 1966). The antibacterial activity of these extracts was studied at the Department of Biology, Institute of Biology and Earth Sciences, Pomeranian University in Słupsk (Poland). In the current study, two *Enterococcus faecalis* strains were used, i.e. *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ (resistant to vancomycin; sensitive to teicoplanin) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™. The strains were inoculated onto Mueller-Hinton (MH) agar plates. Sterile filter

paper discs impregnated with extracts were applied over each of the culture plates. Isolates of bacteria were then incubated at 37 °C for 24 h. The plates were then observed for the zone of inhibition produced by the antibacterial activity of ethanolic extracts derived from the leaves of *A. commutatum* and its cultivars (Malay Beauty, Silver Queen, Silver King). A control disc impregnated with sterile 96% ethanol was used in each experiment. At the end of the period, the inhibition zones formed were measured in millimeters using the vernier. For each extract, eight replicates were assayed (n = 8). The plates were observed and photographs were taken. The susceptibility of the test organisms to the plant extracts was indicated by a clear zone of inhibition around the paper discs containing the plant extracts and the diameter of the clear zone was taken as an indicator of susceptibility. Zone diameters were determined and averaged. The following zone diameter criteria were used to assign susceptibility or resistance of bacteria to the phytochemicals tested: Susceptible (S) ≥15 mm, Intermediate (I) = 10–15 mm, and Resistant (R) ≤10 mm (Okoth et al., 2013).

Statistical analysis

Zone diameters were determined and averaged. Statistical analysis of the data obtained was performed by employing the mean ± standard error of the mean (S.E.M.). All variables were randomized according to the phytochemical activity of the extracts tested according to Okoth et al. (2013). All statistical calculation was performed on separate data from each strain. The data were analyzed using a one-way analysis of variance (ANOVA) using Statistica v. 13.3 software (TIBCO Software Inc., Krakow, Poland) (Zar, 1999).

Results and discussion

The ability of the selected ethanolic plant extracts derived from leaves of *A. commutatum* and its cultivars to inhibit the growth of two *E. faecalis* strains, i.e. *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ (resistant to vancomycin; sensitive to teicoplanin) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™ was determined in this study. The results revealed that four extracts exerted antibacterial activity against these strains. Moreover, the extracts derived from leaves of *A. commutatum* and cv. Silver Queen exhibited higher inhibitory activity ($p < 0.05$) against the growth of *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ than the extracts from cv. Melay Beauty and Silver King. Maximum *in vitro* inhibition was scored by cv. Silver Queen, followed by *A. commutatum*, cv. Malay Beauty and Silver King, which presented inhibition zones of (14.52 ±1.07) mm, (14.34 ±0.98) mm, (13.71 ±1.10) mm, and (13.29 ±1.16) mm, respectively. In the case of the positive controls, 96% ethanol possesses a mild anti-*E. faecalis* effect, which presented inhibition zones of (9.15 ±0.99)

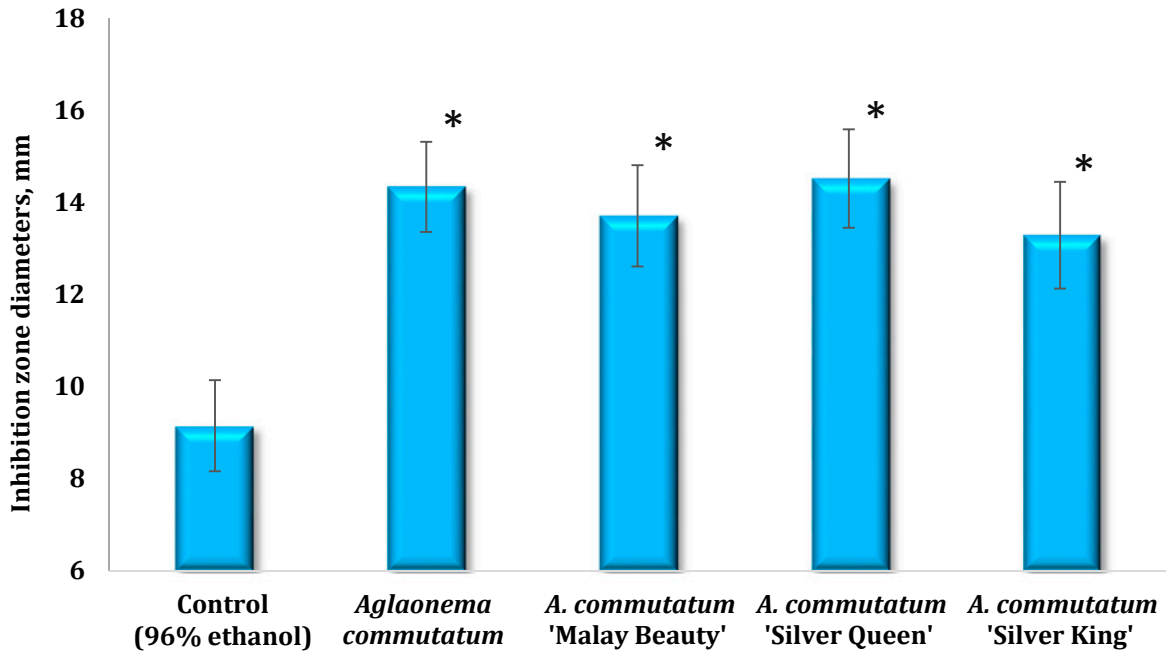

Figure 1 Antimicrobial activity of various extracts derived from leaves of *Aglaonema commutatum* Schott and its cultivars against *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ strain by disc diffusion method
*denote significant differences between the control and extract-treated groups ($p < 0.05$)

mm. The increase in inhibition zone diameters was recorded by 58.7% ($p < 0.05$) for the cv. Silver Queen, 56.7% ($p < 0.05$) for *A. commutatum*, 49.8% ($p < 0.05$) for the cv. Malay Beauty, and 45.3% ($p < 0.05$) for the cv. Silver King compared to the 96% ethanol samples (Figure 1).

Detailed photos regarding the zones of inhibition by the various plant extracts derived from leaves of *A. commutatum* and its cultivars against *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ strain were recorded and presented in Figure 2.

Assessment of antibacterial activity of extracts derived from leaves of *A. commutatum* and its cultivars against the growth of *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™ strain revealed that these extracts exerted antibacterial activity against this strain. Extracts derived from leaves of *A. commutatum* cv. Melay Beauty and Silver Queen exhibited higher inhibitory activity ($p < 0.05$) against the growth of *E. faecalis* than the extracts from the cv. Silver King and *A. commutatum*. Maximum *in vitro* inhibition was scored by cv. Malay Beauty, followed by cv. Silver Queen and Silver King, and *A. commutatum*, which presented inhibition zones of (15.74 ±1.02) mm, (14.55 ±0.97) mm, (13.64 ±0.90) mm, and (11.55 ±0.88) mm, respectively. In the case of the positive controls, 96% ethanol possesses a mild anti-*E. faecalis* effect, which presented inhibition zones of (8.92 ±0.91) mm. The increase in inhibition zone diameters was recorded by 76.5% ($p < 0.05$) for the cv. Malay Beauty, 63.1% ($p < 0.05$) for cultivar Silver Queen, 52.9% ($p < 0.05$) for the cv. Silver King, and 29.5% ($p > 0.05$) for the *A. commutatum* compared to the 96% ethanol samples (Figure 3).

Detailed photos regarding the zones of inhibition by the various plant extracts derived from leaves of *A. commutatum* and its cultivars against *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™ strain were recorded and presented in Figure 4.

In line with our previous studies according to the antibacterial potential of different tropical and subtropical plants, in the current study, we examined

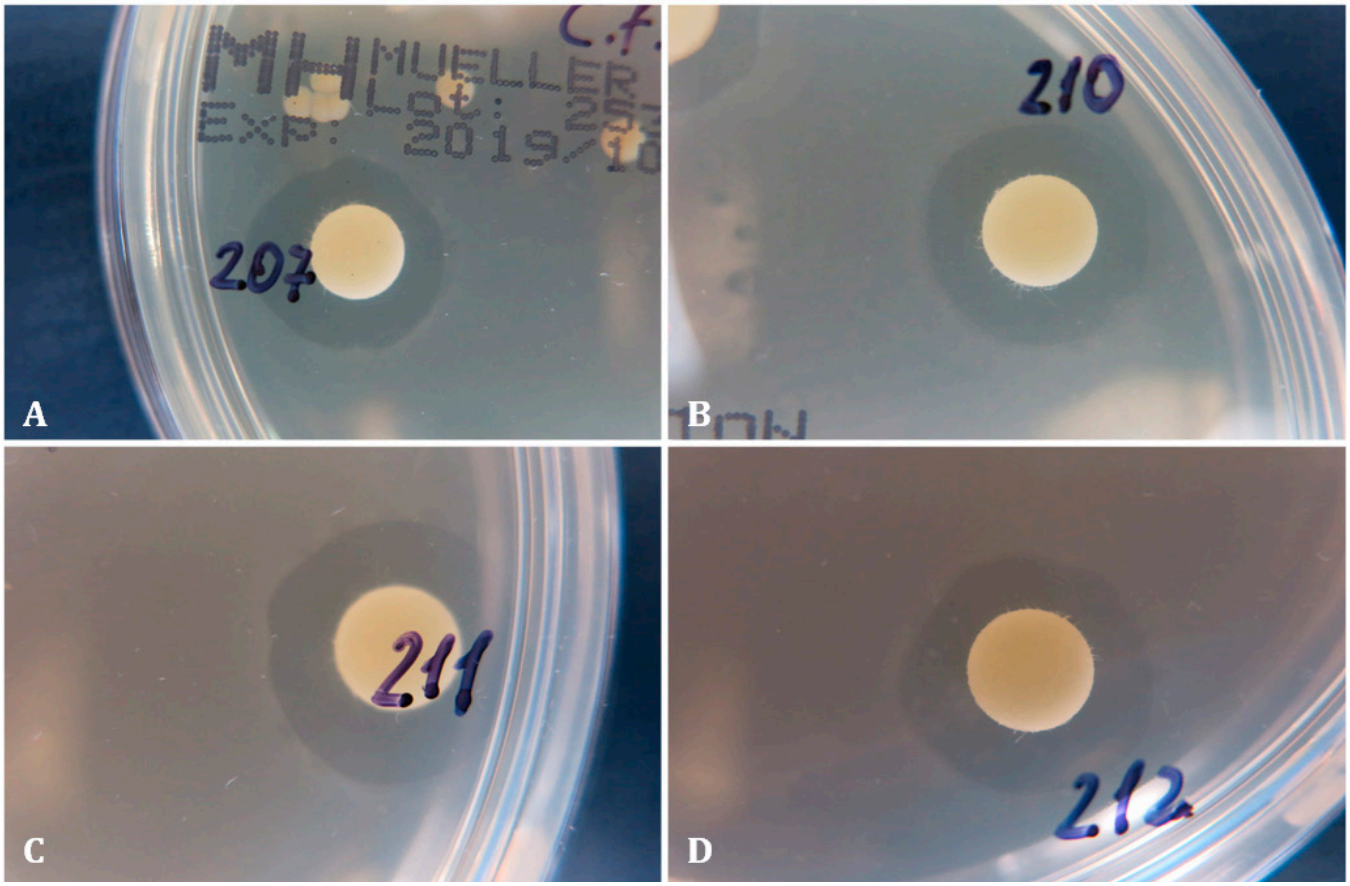

Figure 2 Inhibition growth zones induced by various ethanolic extracts derived from leaves of *Aglaonema commutatum* Schott (A) and cultivars Malay Beauty (B), Silver Queen (C), Silver King (D) against *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ strain

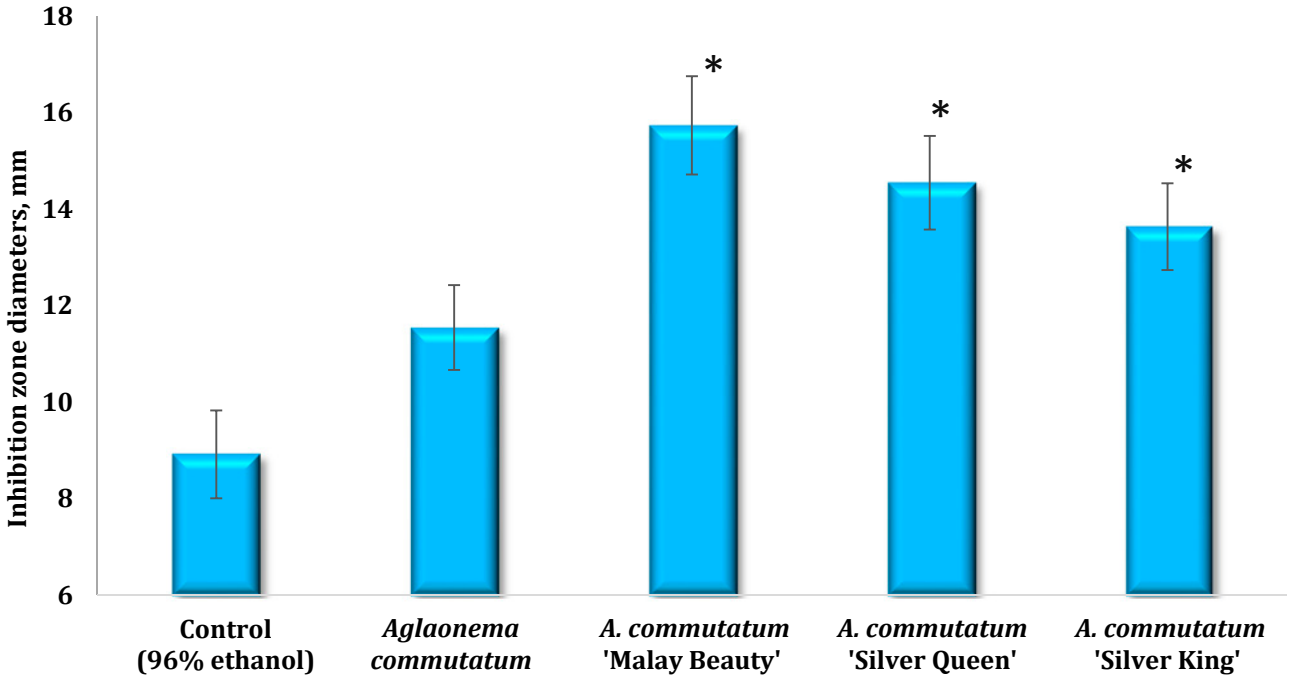

Figure 3 Antimicrobial activity of various extracts derived from leaves of *Aglaonema commutatum* Schott and its cultivars against *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™ strain by disc diffusion method
* denote significant differences between the control and extract-treated groups ($p < 0.05$).

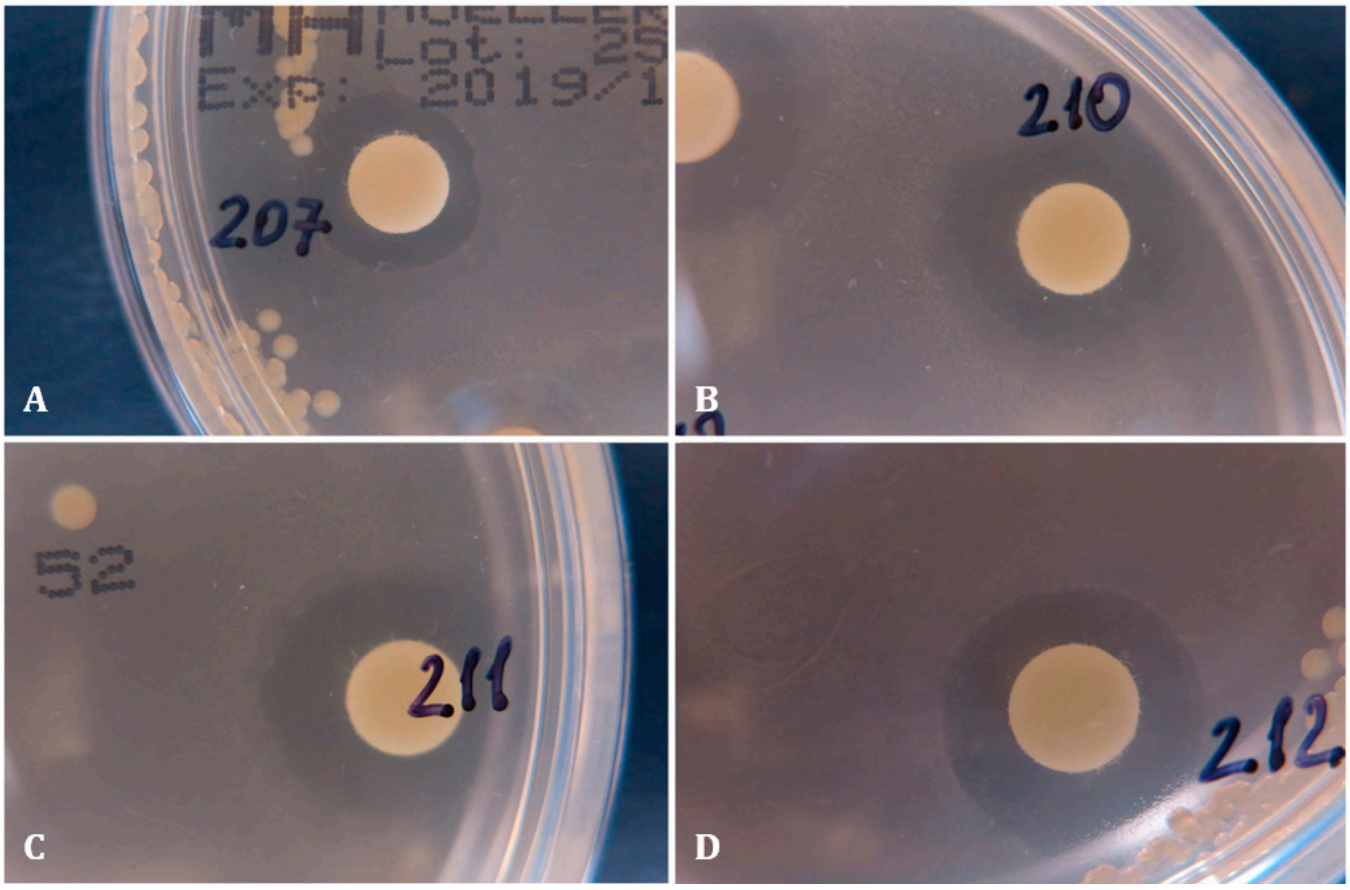

Figure 4 Inhibition growth zones induced by various ethanolic extracts derived from leaves of *Aglaonema commutatum* Schott (A) and cultivars Malay Beauty (B), Silver Queen (C), Silver King (D) against *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™ strain

the antibacterial properties of ethanolic extracts derived from leaves of *A. commutatum* and its cultivars against two *E. faecalis* strains, i.e. *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ (resistant to vancomycin; sensitive to teicoplanin) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™ using the disc diffusion method. The lowest antibacterial activity was presented by extract derived from leaves of *A. commutatum* against *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™ strain (Figure 3). The largest diameter of zone inhibition was observed for ethanolic extracts derived from *A. commutatum* cv. Silver Queen and Melay Beauty (Figure 1 and 3).

In our previous study (Opryshko et al., 2019), we focused on investigating the *in vitro* antibacterial activity of ethanolic extracts derived from leaves of *A. commutatum* and its cultivars (Malay Beauty, Silver Queen, and Silver King), cultivated under glasshouse conditions at M.M. Gryshko National Botanic Garden, against *Citrobacter freundii* strain locally isolated from human materials. The extracts derived from leaves of *A. commutatum* and cultivars Silver Queen exhibited higher inhibitory activity ($p < 0.05$) than the extracts from cv. Melay Beauty and cv. Silver King. The highest *in vitro* inhibition was scored by *A. commutatum*, followed by cv. Silver Queen, Malay Beauty, and Silver King (Opryshko et al., 2019).

Antimicrobial activities of various ethanolic extracts derived from leaves of *A. commutatum* plants and its cultivars (Malay Beauty, Silver Queen, and Silver King) against *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) strain were screened in our other study. Results obtained in this study revealed that the leaf extracts derived from *A. commutatum* cv. Silver Queen and Silver King exhibited higher inhibitory activity than the extracts from *A. commutatum* and *A. commutatum* cv. Melay Beauty. Maximum *in vitro* inhibition was scored by *A. commutatum* cv. Silver Queen, followed by *A. commutatum* cv. Silver King, *A. commutatum*, and *A. commutatum* cv. Malay Beauty (Opryshko et al., 2020a). Another our study aimed to evaluate the antibacterial activity of ethanolic extracts obtained from *A. commutatum* and its cultivars against *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC® 27853™) strain (Opryshko et al., 2020b). The results obtained revealed that two extracts exerted antibacterial activity against *P. aeruginosa* strain. However, the extracts derived from leaves of *A. commutatum* and cv. Silver Queen exhibited higher inhibitory activity than the extracts of cv. Melay Beauty and Silver King. Maximum *in vitro* inhibition was scored by *A. commutatum* cv. Silver Queen, followed by *A. commutatum*, *A. commutatum* cv. Malay Beauty and Silver King, which presented inhibition zones of (11.4 ±1.0) mm, (10.2 ±1.8) mm, (9.5 ±0.9) mm, and (8.5 ±0.9) mm, respectively (Opryshko et al., 2020b).

Some information is available concerning the antimicrobial activity of the studied plant species. For example, Roy et al. (2011) have screened phytochemical substances to assay cytotoxicity and antibacterial activities of ethanolic extracts of leaves of two medicinal plants, *Aglaonema hookerianum* Schott (Family: Araceae) and *Lannea grandis* Engl. (Family: Anacardiaceae) available in Bangladesh. The brine shrimp lethality bioassay showed that the ethanolic extracts of *Aglaonema hookerianum* and *Lannea grandis* possessed cytotoxic activities with LC_{50} 5.25 ($\mu g.mL^{-1}$) and 5.75 ($\mu g.mL^{-1}$) and LC_{90} 10.47 ($\mu g.mL^{-1}$) and 9.55 ($\mu g.mL^{-1}$), respectively. Two extracts obtained from leaves were examined for their antibacterial activities against some gram-positive bacteria such as *Bacillus subtilis*, *Bacillus megaterium*, and *Staphylococcus aureus*, also gram-negative strains of *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *Salmonella paratyphi*, and *Vibrio cholerae*. The agar disc diffusion method was applied to observe the antibacterial efficacy of the extracts. Results indicated that both plant extracts ($\mu g.mL^{-1}$) displayed antibacterial activity against all of the tested microorganisms. The ethanolic extracts of leaves of *Aglaonema hookerianum* showed significant antimicrobial activity (zone of inhibition: 15.08 ±0.45 mm to 20.37 ±0.45 mm) against all tested bacterial strains and the highest zone of inhibition was observed against *S. paratyphi* (20.37 ±0.45 mm). The ethanolic extracts of *Lannea grandis* leaves also showed significant activity against all tested bacteria with a zone of inhibition ranging from 13.93 ±0.09 mm to 18.25 ±0.54 mm. These results were also compared with the zones of inhibition produced by the commercially available standard antibiotic, Amoxicillin at a concentration of 10 µg per disc. Observed antibacterial properties of the ethanolic extract of *Aglaonema hookerianum* and *Lannea grandis* showed that both plants might be useful sources for the development of new potent antibacterial agents (Roy et al., 2011).

Some *Aglaonema* plants can also be recommended as a potent source of neuroprotective and a libido-boosting drug candidate for the management of neurological and sexual disorders. Pharmacological insights on the antidepressant, anxiolytic and aphrodisiac potentials of methanolic extract derived

from leaves of *Aglaonema hookerianum* Schott. (MEAH) in Swiss albino mice were done by Goni et al. (2021). Swiss albino mice (20–30 g) were orally administrated with MEAH at doses ranging from 100 to 400 mg.kg^{-1}, b.w. The elevated plus maze (EPM) and hole board test (HBT) were performed to determine the anxiolytic activity and the forced swimming test (FST) and tail suspension test (TST) were performed to determine the antidepressant activity of MEAH. Besides, the aphrodisiac activity of MEAH was conducted through mounting behaviour and orientation behaviour analysis. Diazepam (1 mg.kg^{-1}, b.w., i.p.) for EPM and HBT; fluoxetine HCl (20 mg.kg^{-1}, b.w., p.o.) for FST and TST, and sildenafil (5 mg.kg^{-1}, b.w., p.o.) for the mounting behaviour analysis and orientation behaviour analysis were used as reference drugs. Goni et al. (2021) revealed that the administration of the MEAH produced strong dose-dependent anxiolytic effects in both HBT and EPM tests. Likewise, the extract showed a significant reduction in the immobility time in both FST and TST compared to the control group. Besides, the MEAH was also found to possess marked aphrodisiac activity complying with several facets such as an increase in sexual performance at the highest dose (400 mg.kg^{-1}, p.o.) and the orientation toward female mice at all tested doses.

Aglaonema plants are one of the potential sources of phytochemicals for the treatment of atherosclerosis. The *Aglaonema* genus contains polyhydroxy alkaloids that exhibit glycosidase inhibitor activity. Ismail et al. (2017) have reported a phytochemical screening of *in vitro Aglaonema simplex* plantlets and the potential compounds as alternatives of SR-B1 ligands that play a role in reducing atherosclerosis. The phytochemical screening was conducted using thin-layer chromatography and attenuated total reflectance-Fourier transform infrared spectroscopy on methanol crude extracts of leaves, stems, and roots. SR-B1 ligand activities were tested on HepG2 cell line stably transfected with SR-B1 promoter. The results showed that the extracts contained secondary metabolites belonging to terpenoids, steroids, phenolics, alkaloids, and glycosides. Luciferase assay suggested that the stem and root extracts increased the expression of SR-B1 at 1.61- and 1.72-fold higher than the control, respectively (Ismail et al., 2017).

The wide variety of iminosugars present in *Aglaonema* sp. (Araceae), including homonojirimycin (HNJ), homomannojirimycin (HMJ), 2,5-dideoxy-2,5-imino-d-mannitol (DMDP), etc., makes these plants an interesting natural source of these bioactive (Asano et al., 1998, Asano et al., 2005; Rodríguez-Sánchez et al., 2016). HNJ exerted protection against influenza virus infection and produced effective immune responses *in vivo*, as revealed in Zhang and co-workers (2013a) study. HNJ was found to improve the survival rate, prolong the mean survival time, and reduce virus yields in lungs on days 4 and 6 post-infection (p.i.), after the agent had been orally administered to the mice from 2 days before infection to 6 days p.i. Administration of HNJ (1 mg.kg^{-1}) significantly increased interferon (IFN)-γ and interleukin (IL)-10 levels but decreased tumor necrosis factor (TNF)-α and IL-6 levels in serum and lungs of influenza-infected mice on days 2, 4 or 6 p.i. (Zhang et al., 2013a). HNJ showed strong antiviral activity against influenza A/PR/8/34 virus (H1N1) as measured by cytopathic effect reduction assay (Zhang et al., 2013b).

Conclusions

The present study has demonstrated that extracts derived from the leaves of *A. commutatum* and its cultivars exhibited antibacterial activity against two *E. faecalis* strains, i.e. *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ (resistant to vancomycin; sensitive to teicoplanin) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™. Ethanolic extracts derived from leaves of *A. commutatum* and the cv. Silver Queen proved to be the most effective against *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 51299™ strain. Leaf extracts derived from cultivars such as Melay Beauty, Silver Queen, and Silver King exhibited significant antibacterial activity against *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz 29212™. Therefore, this plant can be used to treat various diseases caused by *E. faecalis* strains. There is a lot of potential for this plant to treat infections caused by these bacteria, such as wound infections, bloodstream infections, and urinary tract infections. However, further research is required to understand the mechanisms involved in the antimicrobial activity of this plant for extensive clinical usage.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

Funding

The authors would like to extend their sincere appreciation to The International Visegrad Fund for supporting our study.

Acknowledgements

This work was supported by Pomeranian University in Słupsk (Poland) in cooperation with M.M. Gryshko National Botanic Garden, National Academy of Science of Ukraine (Kyiv, Ukraine).

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

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Comparative study of biochemical composition of *Paulownia tomentosa* (Thunb.) Steud. genotypes

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Article Details:
Received:2022-07-10
Accepted:2022-08-09
Available online:2022-11-30

DOI: https://doi.org/10.15414/ainhlq.2022.0019

The review of previous studies on *Paulownia* spp. showed that these plants are valuable raw materials with polyfunctional use among which are medicinal, forage, energetic, etc. This study demonstrated the accumulation of selected biochemical components in the different parts of *Paulownia tomentosa* (Thunb.) Steud. genotypes plants by the end of vegetation collected from experimental collections of M. M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine. The accumulation of selected nutrients in the leaves was the following: a dry matter of 24.09–29.44%, lipid content of 5.01–8.58%, total sugar content of 5.51–9.82%, mono sugar content of 1.73–6.17%, ash content of 1.09–8.96%, phosphorus content of 0.47–1.60%, calcium content of 1.41–3.43%, and heating value of raw material of 4,083.09–4,353.11 Kcal.kg^{-1}. In the branches of investigated genotypes on average accumulated 37.5–46.0% of dry matter, 3.69–6.52% of lipids, 6.66–19.96% of total sugar content, 1.95–5.75% of mono sugar content, 1.43–2.93% of ash content, 0.38–0.89% of phosphorus content, 0.515–1.61% of calcium content, and 3,911.45–4,290.78 Kcal.kg^{-1}of heating value. The trunks had 40.09–51.5% of dry matter, 2.0–6.14% of lipids, 6.44–20.48% of sugars, 1.6–3.67% of mono sugars, 1.18–2.53% of ash, 0.22–0.40% of phosphorus, 0.37–0.63% of calcium, and 4,073.45–4,525.28 Kcal.kg^{-1} of heating value. A very strong correlation was found between sugars and mono sugars content in the leaves (r = 0.859), lipids and phosphorus (r = 0.864) in the branches, heating value, and calcium (r = 0.820) in the trunks. Due to the increasing interest in the growth and use of *P. tomentosa* during the last time, this study can be useful for further breeding work with this species as biofuel, forage, and medicinal plants.

Keywords: *Paulownia*, sugars, lipids, ash, heating value, correlation

Introduction

Paulownia tomentosa (Thunb.) Steud. belongs to the Paulowniaceae Nakai family, although numerous authors attribute this genus to Scrophulariaceae Juss. (Xia et al., 2019). This species is native to China and was introduced to Central Europe in 1834 as an ornamental plant (Kiermeier, 1977). This is a fast-growing and multi-purpose agroforestry tree, the leaves of which are used for domestic animal feeding and exhibit an antimicrobial effect (Bodnar et al., 2020). This tree is one of the few with a C_4 path of photosynthesis and its leaves can be used as green fertilizer (Woźniak et al.,

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2018). *Paulownia* tree is a good adapted to a wide range of climate and soil conditions and possesses some indicators of invasiveness (Essl, 2007; El-Kader and El-Ghit, 2015). But this tree is used by horticulturalists and gardeners as an ornamental plant due to its attractive lavender blossoms (Snow, 2015). This is a deciduous tree with medicinal properties. Leaves, flowers, roots, and seeds in different countries such as China, and Korea are used in folk herbal medicine to treat hemorrhoids, carbuncles, inflammatory bronchitis, gonorrhea, bacteriological diarrhea, etc. Seeds can be used for diabetic complications (He et al., 2016). According to Singh et al. (2018), the leaves of this tree contain ursolic acid and matteucinol, and raw demonstrates the cardioprotective and antioxidant potential.

Essential oil of fresh flowers of *P. tomentosa* exhibited antimicrobial activity and contains numerous compounds among which geranyl, geraniol, nonanal, heptadecane, pentacosane, etc. (Ibrahim et al., 2013). Also, the presence of flavonoids raw in these plants exhibited anticancer (Moon and Zee, 2001), antiradical, antioxidant (Shneiderová et al., 2013; He et al., 2016), and immunological (Yang et al., 2019) activities. Fruits of *P. tomentosa* demonstrated an anti-inflammatory effect (Ryu et al., 2017). *Paulownia* nitrogen content can be comparable with some leguminous that allows for use as green crops by farmers in China (Yadav et al., 2013), leaves of *P. elongata* can accumulate up to 17.5% of proteins (Stewart et al., 2018; Al-Sagheer et al., 2019). Among essential amino acids prevailed histidine (4.8% of crude protein), leucine (4.6%), phenylalanine (4.4%), valine (4.3%), and among nonessential amino acids prevailed proline (13.6%) (Al-Sagheer et al., 2019). A tree has a large size of inflorescences and these plants relate to honey species (Yadav et al., 2013). *P. tomentosa* raw rich in dietary flavonoids and fruit extracts of it can reduce blood pressure (Shneiderová and Šmejkal, 2015). According to Stewart et al. (2018), lignin content in the leaves was 10–22%, and the lowest content of lignin was found in *P. elongata*. Polysaccharides from this plant exhibited immunomodulatory activity (Chen et al., 2021).

Due to the high productivity of this plant, it can be used for biofuel goals (Rodríguez-Seoane et al., 2020; Jakubowski, 2022). The wood chemical composition of *P. tomentosa* (up to 3 years) showed 40% of cellulose, 36% of hemicellulose, and 24% of lignin content (Esteves et al., 2021).

Along with other tree *Paulownia* species can be used for phytoremediation purposes due to their tolerance to high concentrations of metals (Drzewiecka et al., 2021). In total, the sequential biorefinery of plant raw of *Paulownia* species allows using different plant parts for various purposes such as medicinal, biofuel, forage, etc. (Rodríguez-Seoane et al., 2020). According to Youseff et al. (2020), the optimization of micropropagation of *P. tomentosa* using proline can improve the salinity tolerance of this plant.

This study aimed to determine the biochemical composition of different parts of *P. tomentosa* plants of various genotypes as a potential source of raw material for energetic value.

Material and methodology

Plant material

The fresh leaves, branches, and trunks of *Paulownia tomentosa* Steud. genotypes such as f. PSA, f. PL, f. PB, f. PN, f. PO, f. PKS were studied. Plant raw was collected from an experimental collection of the Cultural Flora Department of M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine in October 2020–2021.

Biochemical analysis

Dry matter determination

Plant samples were dried in a drying oven at 105 °C till constant weight in aluminum boxes. Results are given in percentages (Hrytsajenko et al., 2003).

The total content of sugars and mono sugars determination

The total content of sugars was investigated by Bertrand‘s method in water extracts. 4 g of fresh mass was mixed and homogenized with distilled water (approximately 50 ml) in the 100 ml test-tubes and heated in the water bath at 70 °C during 15–20 min. After cooling in the obtained mixtures added 1 ml of the phosphate-oxalate mixture. After this was added 1.5 ml of lead acetate. The obtained mixture brings to the mark (100 ml) with water. After filtration from obtained solution took 50 ml and mixed with 8 ml of 20% HCl (at 70 °C in a water bath for 5 min) after cooling was neutralized by 12% NaOH and brought to the mark by distilled water (100 ml). 3 ml of the obtained solution was mixed with 6 ml of Fehling's solution reagent (6 min boiling in the water bath). The obtained mixture was analyzed for the total content of sugars. The monosugar content was determined from the solvent without the inversion procedure following adding Fehling's solution reagent. In this case, 3 ml

of water extracts were mixed with 6 ml of reagent (Fehling's solution). The following procedure is the same as with the total content of sugars. Results are given by percentages (Hrytsajenko et al., 2003).

The total content of lipids

The total content of lipids is determined in the Soxhlet apparatus (Yermakov et al., 1972). The low-boiling petroleum ether (40 °C) was used as an extractor. The difference in masses before and after the extraction process is used to calculate the total lipid content.

The heating value of raw

The procedure of caloricity measurement was conducted using a calorimeter IKA C-200 (Germany). 0.1–0.2 g of dried plant raw material was combusted in an oxygen bomb for approximately 15 minutes.

The total content of ash

The total content of ash is determined by combustion in the muffle oven at 200–500 °C for 3 days considering the mass before and after combustion (Hrytsajenko et al., 2003).

Statistical analysis

The results are expressed as mean values of three replications ± standard deviation (SD); hierarchical cluster analyses of similarity between samples were computed based on the Euclidean similarity index. Data were analyzed with the ANOVA test and differences between means were compared through the Tukey-Kramer test ($p < 0.05$).

Results and discussion

The study of the biochemical composition of the whole crop and selected parts of the plant should be considered in the evaluation of raw. The partitioning of biomass may significantly change the biofuel quality, for example, stems of switchgrass (*Panicum virgatum* L.) and *Miscanthus* spp. showed better higher biochemical component content than leaves (Monti et al., 2008). The biochemical composition of plants, especially dry matter content, ash content, mineral composition, and calorific value of energetic plants is a very important parameter for evaluating raw (Vergun et al., 2022). One of the widely used parameters of plant species for energetic purposes is dry matter content. This parameter depends on the period of growth and dry matter accumulated during the vegetation period.

In this study, the dry matter content of leaves, branches, and trunks of *P. tomentosa* genotypes was 24.09–29.44%, 37.5–46.0%, and 40.09–51.5%, respectively, depending on genotypes (Figure 1). As showed the results, the highest content of dry matter was found in the trunks and the lowest in the leaves.

According to Al-Sagheer et al. (2019), paulownia leaf meal had 88.12% of dry matter. The dry matter content closely relates to total biomass productivity and some growth characteristics (Greco and Cowagnaro, 2005).

Lipids are one of the most important components of plant cells and act as signaling and energy storage compounds (Suh et al., 2015; Hou et al., 2016). The content of lipids in the plant is one of the most essential parameters that determine the nutritional value of raw and varies depending on the species and part of the plant (Vergun et al., 2017; Vergun et al., 2020).

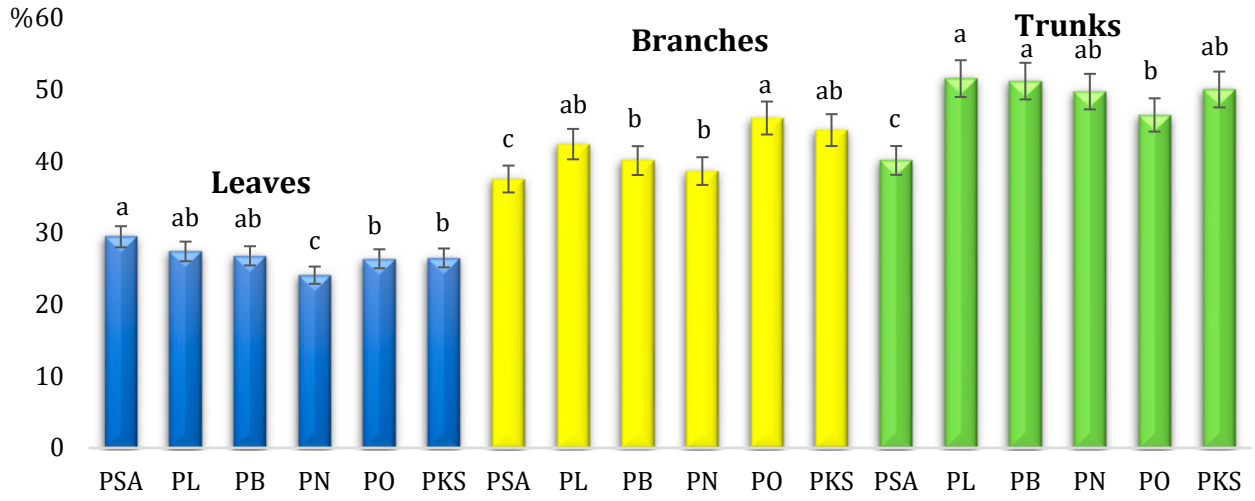

Figure 1 The dry matter content of raw *Paulownia tomentosa* (Thunb.) Steud. genotypes at the end of vegetation; different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

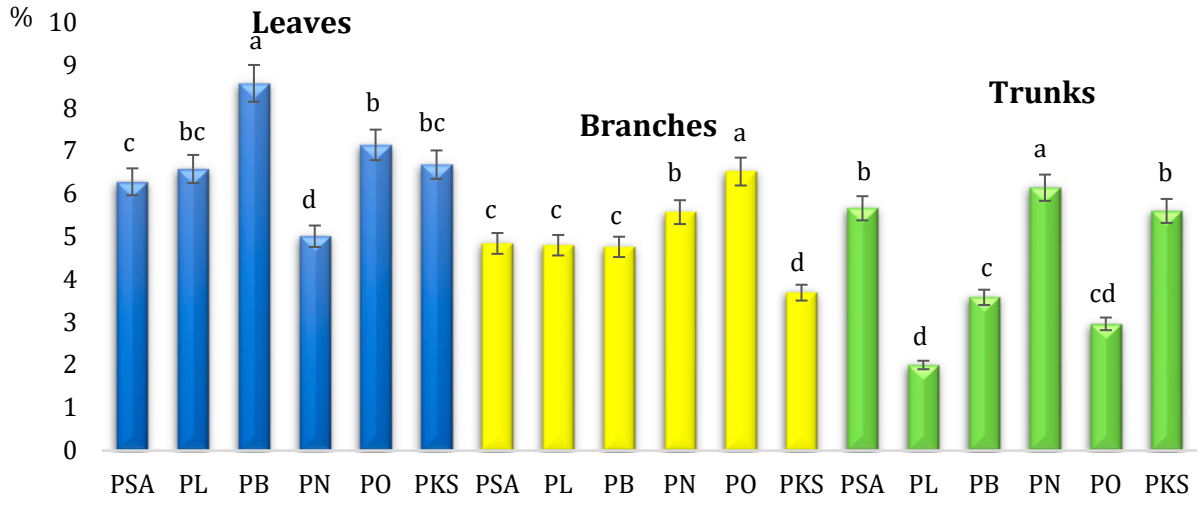

Figure 2 The lipid content of raw *Paulownia tomentosa* (Thunb.) Steud. genotypes at the end of vegetation; different superscripts in each column indicate the significant differences in the mean at p <0.05

The content of lipids in raw *P. tomentosa* genotypes was from 5.01 to 8.58% in the leaves, from 3.69 to 6.52% in the branches, and from 2.0 to 6.14% in the trunks (Figure 2).

According to Stewart et al. (2018), the content of lipids was from 1.9 to 3.8%, and in leaves 2.87%. Angelova-Romova et al. (2011) determined the content of oil in the seeds of *P. tomentosa* of 20.3%.

Sugars play an important role in plant metabolism and they are substrates of energetic processes. They also play a regulatory role in photosynthesis and modulate gene expression (Eckstein et al., 2012). The sugars also influence plant growth (Onto et al., 2001; Lastdrager et al., 2014). According to Ende (2014), a minimal sucrose dose is required for lateral bud outgrowth.

The level of sugars in plant parts depends on complex factors such as conditions of growth, stress factors of the environment, and physiological peculiarities of development (Ciereszko, 2018). The total content of sugars in leaves of *P. tomentosa* was 5.51–9.82%, in the branches 6.66–19.96%, and 6.44–20.48% in the trunks depending on genotypes (Figure 3).

According to Rakhmetova et al. (2020), the total content of sugars in the above-ground part of another energetic plant *Panicum virgatum* was 4.44–9.15% depending on genotype and stage of growth.

Along with the total content of sugars was studied mono sugars content in different organs of investigated plants (Figure 4). We found 1.73–6.17% of mono

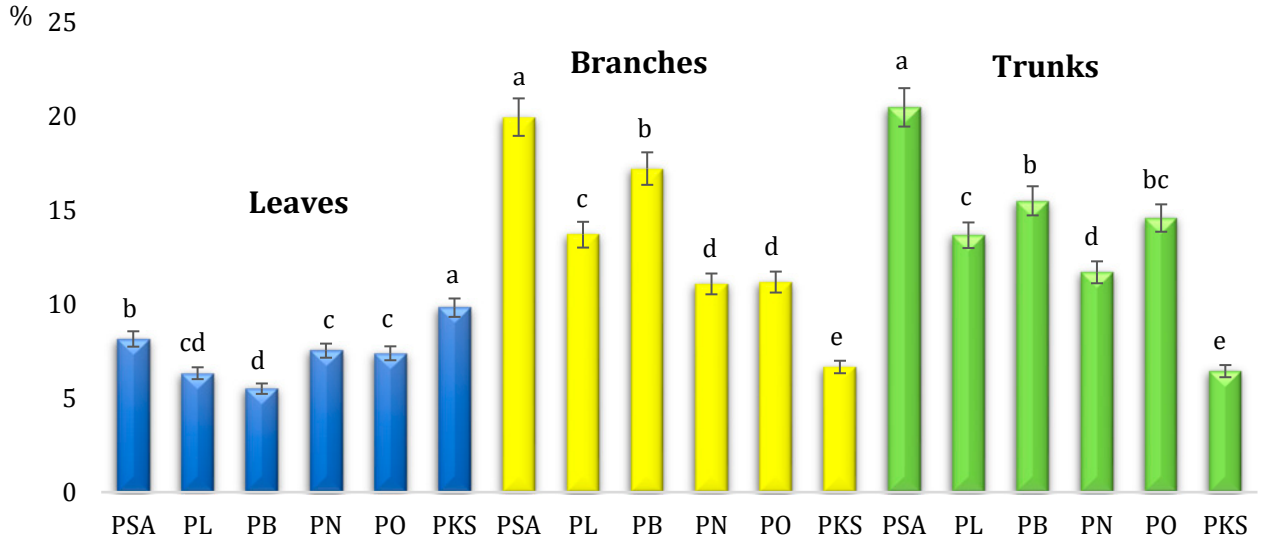

Figure 3 The total sugar content of raw *Paulownia tomentosa* (Thunb.) Steud. genotypes at the end of vegetation; different superscripts in each column indicate the significant differences in the mean at p <0.05

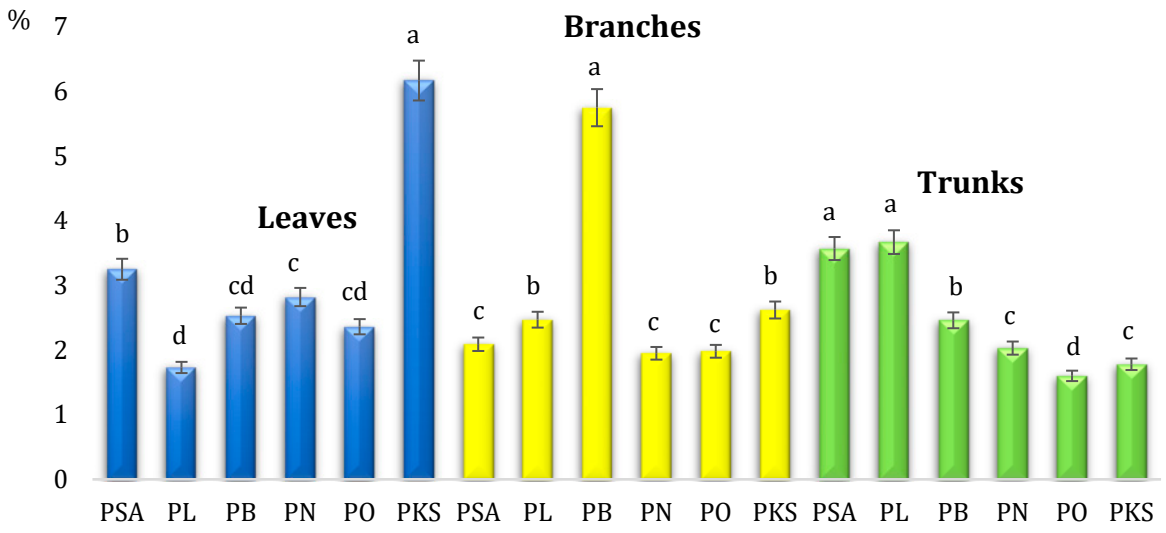

Figure 4 The total mono sugar content of raw *Paulownia tomentosa* (Thunb.) Steud. genotypes at the end of vegetation; different superscripts in each column indicate the significant differences in the mean at p <0.05

sugars in the leaves, 1.95–5.75% in the branches, and 1.6–3.67% in the trunks.

The study of monosaccharide content of *Paulownia fortunei* (Seem.) Hemsl. showed that the most prevailed of them was galactose (Wang et al., 2019).

The study of thermochemical properties of plant raw such as heating value is an important parameter of biofuel evaluation (Senelwa and Sims, 1999). The heating value of leaves of *P. tomentosa* genotypes was in ranges from 4,083.09 to 4,353.11 Kcal.kg^{-1}, from 3,911.45 to 4,290.78 Kcal.kg^{-1} in the branches, and from 4,073.45 to 4,525.28 Kcal.kg^{-1} in the trunks (Figure 5). The recalculation of obtained data allowed us to find results in MJ.kg^{-1}: 17.09–18.22 in leaves, 16.37–17.96 in branches, and 17.05–18.94 in the trunks.

Stewart et al. (2018) found 18.6–19.6 kJ.kg^{-1} of heating value in leaves. According to Yavorov et al. (2015), the heating value of *P. elongata* raw was 17,970 kG.kg^{-1} which corresponds 4,292 Kcal.kg^{-1} and is close to our results. Qi et al. (2016) found 4,521.5, 4,593.3, 4,114.8, and 4,258.4 Cal.g^{-1} of heating value for stems, branches, leaves, and barks, respectively. The heating value of one-year leaves of this species was 15.9–18.7 MJ.kg^{-1} (or 3,798–4,467.4 Kcal.kg^{-1}) as reported Jacek and Litwińczuk (2016).

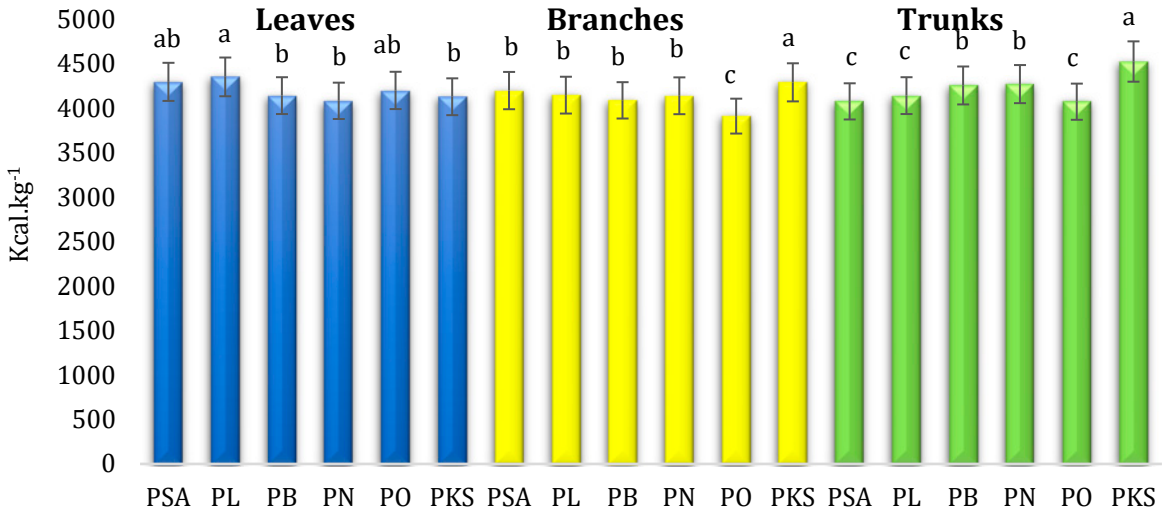

Figure 5 The heating value of raw *Paulownia tomentosa* (Thunb.) Steud. genotypes at the end of vegetation; different superscripts in each column indicate the significant differences in the mean at p <0.05

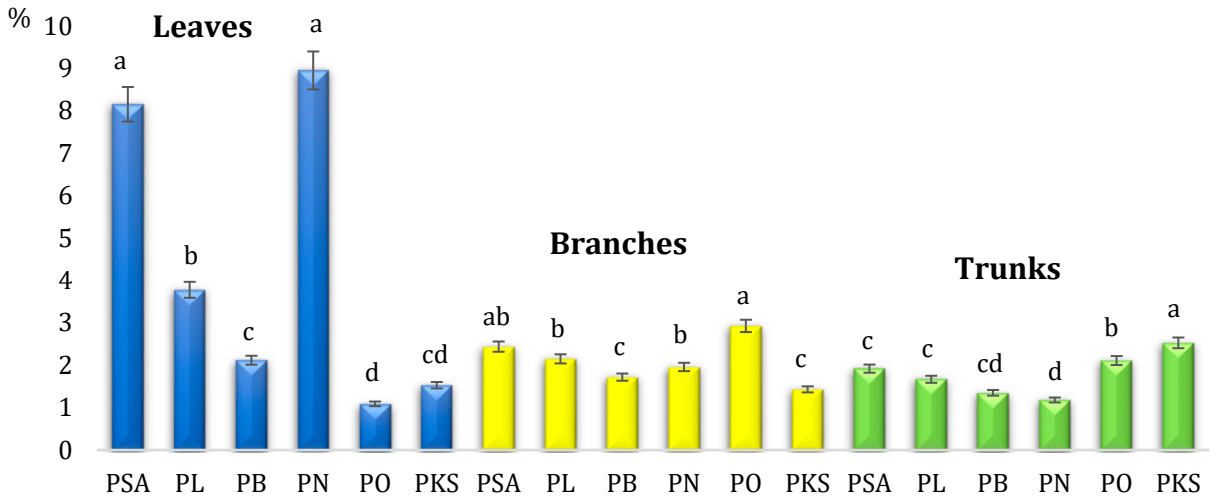

Figure 6 The total ash content of raw *Paulownia tomentosa* (Thunb.) Steud. genotypes at the end of vegetation; different superscripts in each column indicate the significant differences in the mean at p <0.05

One of the important parameters of the nutritional composition of plant raw is the assessment of ash content (Godočiková et al., 2019). The total ash content in raw *P. tomentosa* genotypes was from 1.09 (f. PO) to 8.96 (f. PN) % in the leaves, from 1.43 (f. PKS) to 2.93 (f. PO) % in the branches, and from 1.18 (f. PN) to 2.53 (f. PKS) % in the trunks (Figure 6).

As reported Prochnow et al. (2009), between the heating value and ash content of energetic plants, exists the correlation. Plants with low content of ash have the highest heating value. In this study, minimal values of ash in the leaves were found for f. PO, in the branches for f. PKS, and in the trunks for f. PN. The study of a trihybrid variety of *P. elongata* × *fortunei* × *tomentosa* showed that the content of ash was 8.9 g.kg^{-1} which was less than that of the initial species (López et al., 2012). In our study leaves of plants of f. PO had close value and was the less. According to Yavorov et al. (2015), the ash content of *P. elongata* raw was 1.03% which is close to *P. tomentosa* f. PO leaves in our study. As reported Stewart et al. (2018), the ash content of *P. elongata* during the growth period was from 6 to 9%, and in the leaves 7.67%. According to Al-Sagheer et al. (2019), paulownia leaf meal had 8.85% of ash which was close to our results related to f. PSA and f. PN. Qi et al. (2016) determined ash content in leaves and barks of 6.0 and 2.89%, respectively. As reported Ganchev et al. (2019), the ash content of the leaves of *P. elongata*

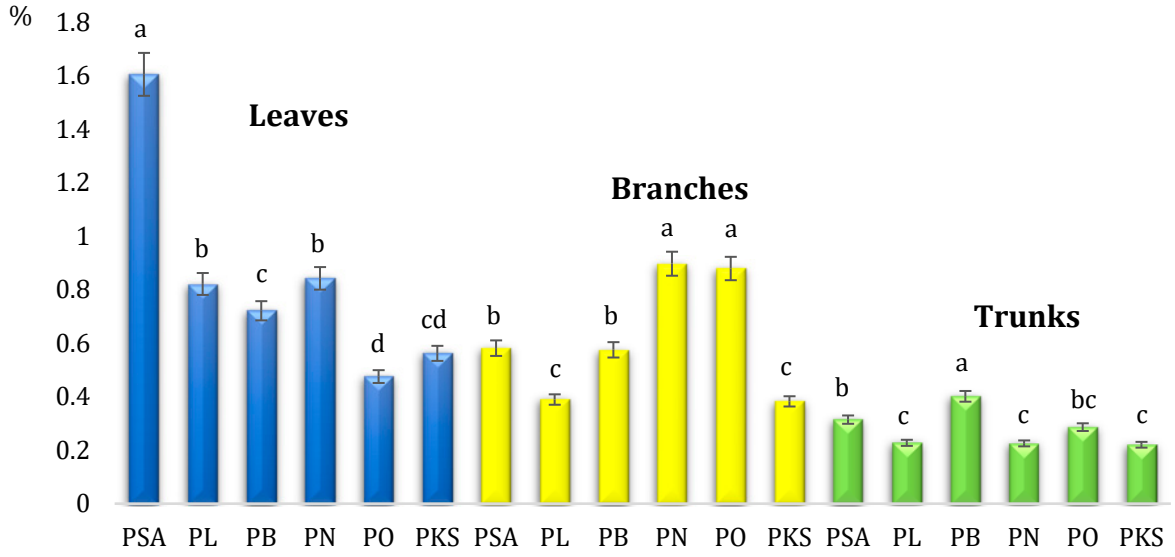

Figure 7 The total phosphorus content of raw *Paulownia tomentosa* (Thunb.) Steud. genotypes at the end of vegetation; different superscripts in each column indicate the significant differences in the mean at p <0.05

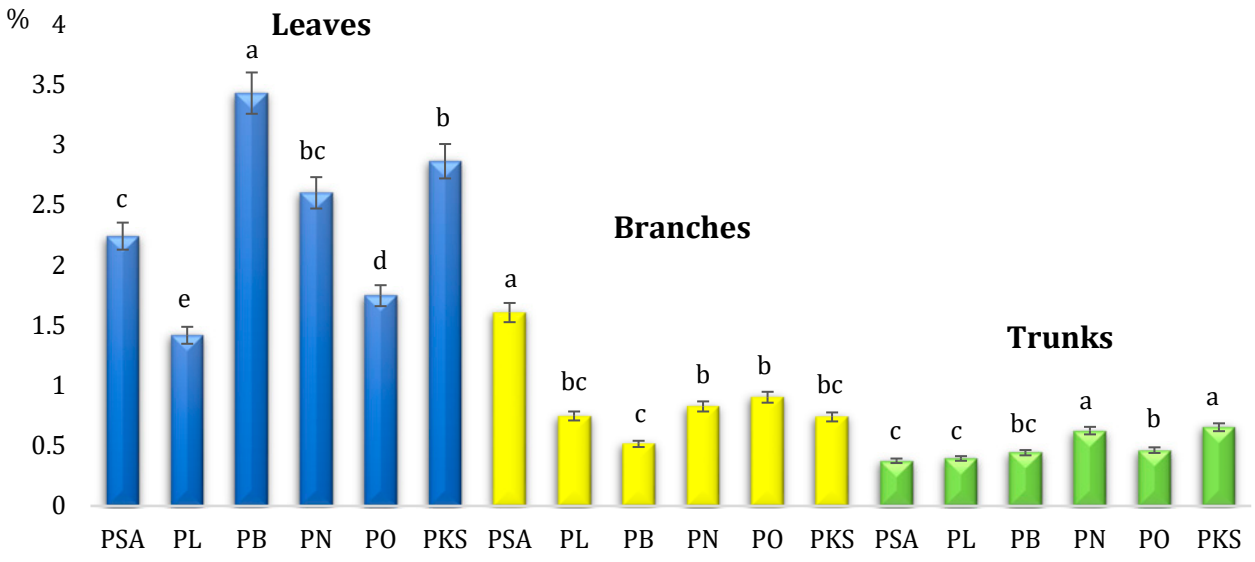

Figure 8 The total calcium content of raw *Paulownia tomentosa* (Thunb.) Steud. genotypes at the end of vegetation; different superscripts in each column indicate the significant differences in the mean at p <0.05

was 14.94% which was higher than in the leaves of *P. tomentosa* in our study. Özelçam et al. (2020) determined the mean value of 9.21% of ash content in the leaves of this plant.

One of the most essential components of ash is phosphorus which plays an important role in enzyme regulation, biosynthesis of nucleic acids, is a key element in some plant physiological processes (like photosynthesis and transpiration), etc. (Lambers, 2022). We determined 0.47–1.60% of phosphorus in the leaves, 0.38–0.89% in the branches, and 0.22–0.40% in the trunks of investigated genotypes of *P. tomentosa* (Figure 7). The minimal values of phosphorus were found in the trunks of investigated plant parts.

Our results were 3–10 times higher compared with Al-Sagheer et al. (2019) who reported about phosphorus content of leaves (0.16%). In the study by Özelçam et al. (2020), this parameter in the leaves of *Paulownia* spp. was on average 0.49% which is close to f. PO in our study.

Calcium is an important nutrient that is required for numerous roles in plant organisms on a cellular level and adequate concentration in plant natural habitats varied from 0.1 to 5% of dry weight (White and Broadley, 2003). The calcium content in the leaves, branches, and trunks of *P. tomentosa* varieties was 1.41–3.43%, 0.515–1.61%, and 0.37–0.63%, respectively (Figure 8). The minimal accumulation of calcium was fixed in the trunks of investigated raw.

As resulted Al-Sagheer et al. (2019), the content of calcium in the leaf meal of *P. tomentosa* was 0.36% which was less than in our study. According to Özelçam et al. (2020), the calcium content in the leaf raw of this plant was 1.74% which is close to the leaves of f. PO in our study.

The study of the relationship of biochemical compound accumulation allows determining the level of relationship of studied parameters. Use of coefficient of Pearson can be used in biochemical studies to describe some regularities considering as many as possible parameters (Ngamdee et al., 2016). In this study, a very strong correlation was found between sugars and mono sugars content in the leaves (r = 0.859), lipids and phosphorus (r = 0.864) in the branches, heating value and calcium (r = 0.820) in the trunks (Table 1). The strong correlation was determined between heating value and dry matter (r = 0.759), ash and phosphorus (r = 0.744), dry matter and phosphorus (r = 0.643) in the leaves, between sugars and phosphorus (r = 0.609), mono sugars and phosphorus (r = 0.600), heating value and dry matter (r = 0.511) in the trunks. A moderate correlation was found between ash and calcium (r = 0.578), sugars and calcium (r = 0.489) in the branches, and between phosphorus and heating value (r = 0.461) in the leaves.

It should be noted that a very strong negative correlation was found between lipid content and heating value (r = -0.903) in the branches, between sugars and calcium content (r = -0.856), sugars content and heating value (r = -0.852) in the trunks, between lipids and ash content (r = -0.741), calcium content and heating value (r = -0.727) in the leaves.

Table 1 Correlation between investigated parameters of *Paulownia tomentosa* (Thunb.) Steud. genotypes

Parameter	Dry matter	Lipids	Sugars	Mono sugars	Heating value	Ash	P
Leaves							
Lipids	0.318*	1	-0.462*	0.097	0.052	-0.741**	-0.299
Sugars	0.021	-0.462*	1	0.097	-0.216	0.058	0.050
Mono sugars	-0.024	0.097	0.859**	1	-0.422*	-0.181	-0.118
Heating value	0.759**	0.052	-0.216	-0.422*	1	0.038	0.461*
Ash	-0.018	-0.741**	0.058	-0.181	0.038	1	0.744**
P	0.643*	-0.299	0.050	-0.118	0.461*	0.744**	1
Ca	-0.225	0.348	0.026	0.447*	-0.727**	-0.046	-0.077
Branches							
Lipids	0.146	1	0.094	-0.292	-0.903**	0.087	0.864**
Sugars	-0.678*	0.094	1	0.330*	-0.107	0.386*	-0.020
Mono sugars	-0.139	-0.292	0.330*	1	-0.038	-0.388*	-0.261
Heating value	-0.359*	-0.903**	-0.107	-0.038	1	0.296	-0.673*
Ash	-0.844**	0.087	0.386*	-0.388*	0.296	1	0.302*
P	-0.076	0.864**	-0.020	-0.261	-0.673*	0.302	1
Ca	-0.423*	0.103	0.489*	-0.545*	0.148	0.578*	0.101
Trunks							
Lipids	-0.361	1	-0.161	-0.234	0.445	0.042	-0.175
Sugars	-0.671*	-0.161	1	0.600*	-0.852**	-0.327	0.609*
Mono sugars	-0.272	-0.234	0.600*	1	-0.466	-0.230	0.104
Heating value	0.511*	0.445	-0.852**	-0.466	1	0.288	-0.303
Ash	-0.268	0.042	-0.327	-0.230	0.288	1	-0.289
P	-0.189	-0.175	0.609*	0.104	-0.303	-0.289	1
Ca	0.397	0.586*	-0.856**	-0.731**	0.820**	0.159	-0.522

Note: ** Correlation is significant at p ≤0.01; * correlation is significant at p ≤0.05

The ratio of different biochemical parameters may be unaffected by geographical variation due to accumulation selectivity (Garten, 1976). But some investigations showed that the correlation coefficient depends on many factors such as species and genotypes, periods of growth, and studied parameters (Singh et al., 2011; Dinc and Unay, 2021).

Conclusions

This study demonstrated comparable biochemical composition of different parts of *Paulownia tomentosa* at the end of vegetation that accumulated high content of dry matter, lipids, total sugar and monosugar content, ash, and their components calcium and phosphorus. The heating value of investigated plant parts not differed significantly but had high values that characterized biofuel plants. Among investigated genotypes were fixed the highest values of investigated parameters: dry matter for leaves f. PB (51.5%), lipids for leaves f. PB (8.58%), total sugar content for trunks f. PSA (20.48%), monosugar content for f. PKS (6.17%), heating value for trunks f. PKS (4,525.28 Kcal.kg^{-1}), ash content for leaves f. PN (8.96%), phosphorus content for leaves f. PSA (1.61%), and calcium content for leaves f. PB (3.43%). A very strong correlation was found between sugars and mono sugars content in the leaves (r = 0.859), lipids and phosphorus (r = 0.864) in the branches, heating value, and calcium (r = 0.820) in the trunks. Due to the increasing interest in the growth and use of *P. tomentosa* last time, this study can be useful for further breeding work with this species as biofuel, forage, and medicinal plants.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

Aknowledgements

This publication was prepared with the active participation of researchers in the International Network AgroBio*Net*.

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

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Dose-dependent alterations in the biomarkers of lipid and protein oxidation in the blood of patients with type 2 diabetes mellitus after *in vitro* incubation with extracts of *Chelidonium majus* L.

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Article Details:
Received:2022-07-18
Accepted:2022-08-11
Available online:2022-11-30

DOI: https://doi.org/10.15414/ainhlq.2022.0020

The role of oxidative stress in the occurrence and development of diabetes mellitus is both critical and pivotal. Several molecular event cascades in different metabolic pathways such as glycolytic, hexosamine, protein kinase C, polyol and advanced glycation end-product pathways have been identified as pro-oxidative processes and are usually up-regulated in diabetics. Consistent with our previous studies, we continue to evaluate the antioxidant potential of great celandine (*Chelidonium majus* L., CM), a representative of the Papaveraceae family, collected from northern parts of Poland using the blood samples of patients with type 2 diabetes mellitus. Therefore, in the present study, oxidative stress biomarkers (2-thiobarbituric acid reactive substances (TBARS), aldehydic and ketonic derivatives of oxidative modification of proteins (OMP)) were used to evaluate the antioxidant properties of the stalk and root extracts of CM in final doses of 5 mg.mL^{-1}, 2.5 mg.mL^{-1}, 1.25 mg.mL^{-1} and 0.63 mg.mL^{-1}. Plant materials were collected from natural habitats on the territory of the Kartuzy district in the Pomeranian province (northern part of Poland). The use of extracts derived from both roots and stalks of CM collected from both urban and rural agglomerations in final doses of 5 mg.mL^{-1}, 2.5 mg.mL^{-1}, and 1.25 mg.mL^{-1} resulted in a significant enhancement of lipid peroxidation in the blood samples. On the contrary, only incubation of blood samples with stalk extracts of CM collected from urban areas at a final dose of 0.63 mg.mL^{-1} resulted in a no-significant decrease in TBARS levels contributing to the protection of lipid structures in membranes. Similar results were obtained by analyzing levels of aldehydic derivatives of oxidatively modified proteins in the blood samples after *in vitro* incubation with the extracts, where final doses of 5 mg.mL^{-1}, 2.5 mg.mL^{-1}, and 1.25 mg.mL^{-1} significantly increased the oxidation process in protein structures. Analysis of levels of ketonic derivatives of oxidatively modified proteins showed that the use of root extracts of CM collected from urban agglomerations in final doses of 2.5 and 1.25 mg.mL^{-1} reduced levels of oxidatively modified proteins, while the use of stalk extracts of CM harvested from urban agglomerations in a final dose of 0.63 mg.mL^{-1} statistically significantly reduced levels of ketonic derivatives of oxidatively modified proteins compared to the control samples. These *in vitro* studies indicate that extracts derived from this plant are a significant source of natural metabolites that could be cytotoxic in final doses of 5 mg.mL^{-1}, 2.5 mg.mL^{-1}, and 1.25 mg.mL^{-1} to the blood of patients with T2DM. Only a final dose of 0.63 mg.mL^{-1} no significantly changed levels of lipid and protein oxidation in the blood samples.

Keywords: great celandine, root and stalk extracts, blood samples, lipid peroxidation, oxidatively modified proteins, type 2 diabetes mellitus

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Introduction

Diabetes mellitus (DM) is a chronic endocrine and metabolic disorder which is underlined by insulin deficiency or insulin insensitivity or both, and characterized by hyperglycemia and vascular complications (micro and macro). Several pathogenic processes are involved in the development of DM. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is the deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of hyperglycemia. Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or nonketotic hyperosmolar syndrome. Diabetic complications result in considerable morbidity and mortality leading to major healthcare delivery costs (*Diabetes Care*, 2011). Although there are several studies to elucidate the molecular mechanisms underlying the development of DM complications, their precise pathophysiology is not completely understood (Forbes and Cooper, 2013).

Similar to several other health conditions such as cancer and neurodegenerative disorders, oxidative stress has been widely linked with the incidence of DM. Several studies have shown that oxidative stress is a key element in the development and progression of DM and its associated complications. Free radicals are active biomolecules that are physiologically generated during metabolic pathways and/or by immune cells (Yaribeygi et al., 2019, 2020). Free radicals have physiological roles in many molecular pathways including those of cellular signalling, synaptic plasticity, memory formation, defence against invader pathogens, cell-cell interactions, cell growth, autophagy, apoptotic processes, and ageing. When free radical generation increases above the physiological range, it overcomes the antioxidant mechanisms of cells and results in oxidative stress. Free radicals are active derivatives of either the oxygen molecule such as reactive oxygen species (ROS: hydroperoxyl, superoxide, hydrogen peroxide, and hydroxyl radicals) and nitrogen molecules such as the reactive nitrogen species (RNS) such as peroxynitrite. Oxidative stress occurs when there is a distortion in the redox balance of the cell, causing damage to membranes and vital biomolecules such as DNA, proteins, and lipids. Oxidative stress has been shown to compromise the two major mechanisms failing during DM which are insulin secretion and insulin action (Giacco and Brownlee, 2010; Angelova et al., 2018).

Antioxidants can act as chain breakers, scavenging chain-initiating radicals like hydroxyl, alkoxyl, or peroxyl, quenching singlet oxygen, decomposing hydroperoxides, and chelating prooxidative metal ions (Scalbert et al., 2005). Epidemiological studies confirm that the incidence of oxidative stress-related conditions is lowered by the use of medical plants rich in compounds possessing high antioxidant activity (Pisoschi et al., 2016). Plants containing antioxidants and antioxidant nutrients play an important role in the prevention of many disorders and diseases. Recent scientific reports show that plants of the Papaveraceae family contain some metabolites possessing antioxidative properties such as alkaloids, polyphenols, and tannins. *Chelidonium majus* L. (CM) (Papaveraceae family), or greater celandine, is an important plant in western phytotherapy and traditional Chinese medicine (Nawrot et al., 2021). Crude extracts of CM as well as purified compounds derived from it exhibit a broad spectrum of biological activities (antioxidant, anti-inflammatory, antimicrobial, antitumoral, analgesic, hepatoprotective, etc.) that support some of the traditional uses of CM. However, herbal medicine also claims that this plant has several important properties which have not yet been scientifically studied. This species is known to produce a broad range of secondary metabolites, ensuring its therapeutic properties (Zielinska et al., 2018). The main constituents of CM responsible for biological properties are isoquinoline alkaloids such as chelidonine, chelerythrine, sanguinarine, coptisine, berberine, allocryptopine, and protopine. They are reported to have anti-inflammatory, antimicrobial, antibacterial, antiviral, immunomodulatory, anticancer, choleretic, hepatoprotective, and analgesic properties (Zielinska et al., 2018). Celandine raw materials exhibited significant differences in the composition of alkaloids and other antioxidant substances in different parts of plants (Seidler-Łożykowska et al., 2016; Krizhanovska et al., 2021).

This study is a continuation of our previous investigations aimed at the assessment of the antioxidative properties of CM using different

cell models. The aim of this study was evaluation the changes in the biomarkers of oxidative stress (2-thiobarbituric acid reactive substances, carbonyl derivatives of oxidative modification of proteins) in the blood samples collected from T2DM patients *in vitro* exposed to different doses of extracts derived from roots and stalks of CM. These plants were collected in urban and rural agglomerations of the Kartuzy district in the Pomeranian province (northern part of Poland).

Material and methodology

Collection of plant materials

Plant materials (*Chelidonium majus* L.) were harvested from natural habitats on the territory of the Kartuzy district (54° 20′ N 18° 12′ E) in the Pomeranian province (northern part of Poland) (Figure 1). The plant collection covered the period from June to July 2020. For our studies, we collected CM plants in phases beginning with flowering (flower buds visible) and full flowering (yellow flowers blooming, young fruits small and developing). Kartuzy is located about 32 kilometres (20 miles) west of Gdańsk and 35 km (22 miles) south-east of the town of Lębork on a plateau at an altitude of approximately 200 meters (656 feet) above sea level on average. The plateau, which is divided by the Radaune lake, comprises the highest parts of the Baltic Sea Plate (http://www.kartuzy.pl/). Plants were collected from urban (n = 5) and rural agglomerations (n = 15) on the territory of the Kartuzy district.

Preparation of plant extracts

Freshly collected roots and stalks were washed, weighed, crushed, and homogenized in 0.1M phosphate buffer (pH 7.4) (in proportion 1 : 19, w/w) at room temperature. The extracts were then filtered and used for analysis. The obtained extracts were stored at -20 °C until use.

Patients with diabetes mellitus type 2 (T2DM) and collection of blood samples

A total of 7 patients with T2DM between 42 and 68 years old were studied. The participants of the study were recruited among patients of non-public Health Care Center U & O Zdrowie – Home-based long-term care (Lębork, Poland). A detailed medical history was taken, and a physical examination was performed on all participants. The Research Ethics Committee of the Regional Medical Commission in Gdańsk (Poland) approved the current study (KB-31/18; KB-21/19). All patients provided written informed consent before the start of the study procedures. Participants included in the current study were selected according to the following criteria: first, they were diagnosed with type 2 diabetes mellitus patients; second, they were free of

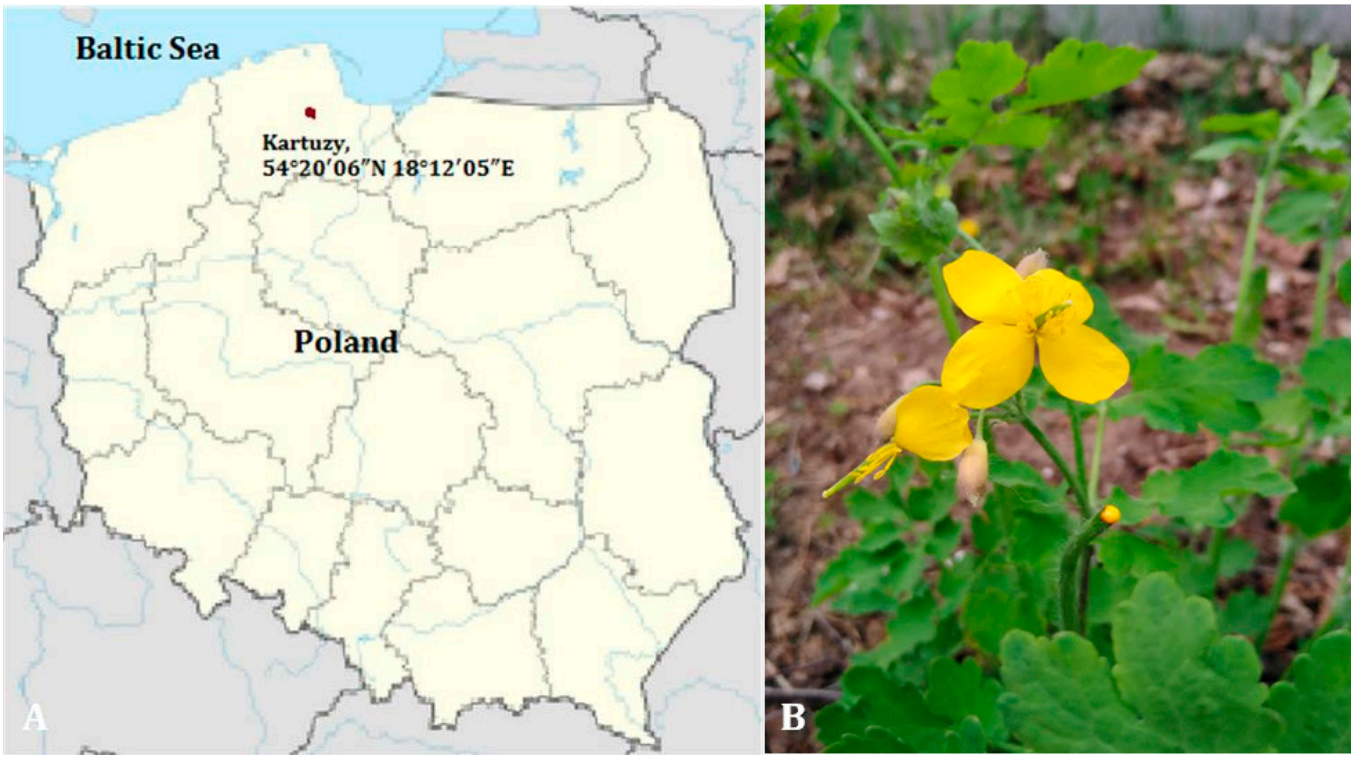

Figure 1 Location of Kartuzy in the map of Poland (A), where the greater celandine (B) was collected

any ailment which could affect the parameters under study. Hemolytic anaemia, haemoglobin variants, hepatic disease, and infectious diseases, such as tuberculosis and sarcoidosis, were excluded from the study.

Blood samples were collected into commercial tubes after overnight fasting for the analysis of laboratory parameters. Venous blood samples (25 ml) were obtained from the capital vein of each participant using sterile disposable plastic syringes. Specimens were collected at the same standardized time to minimize any effect of diurnal variation. The blood samples in the tubes were left to clot and the serum was separated by centrifugation. The clear, non-hemolyzed supernatant sera were separated using clean, dry disposable plastic syringes. Blood samples were stored at +4 °C and used within 2 days for the analysis of biomarkers of oxidative stress.

The 2-Thiobarbituric acid reactive substances (TBARS) assay

The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reacting substances (TBARS) with the Kamyshnikov (2004) method for determining the malonic dialdehyde (MDA) concentration. This method is based on the reaction of the degradation of the lipid peroxidation product, MDA, with 2-thiobarbituric acid (TBA) under high temperature and acidity to generate a coloured adduct that is measured spectrophotometrically. The nmol of MDA per mL was calculated using $1.56 \cdot 10^5$ $mM^{-1}.cm^{-1}$ as the extinction coefficient.

The carbonyl derivatives of protein oxidative modification (OMP) assay

To evaluate the protective effects of extracts derived from roots and stalks of CM collected in urban and rural agglomerations against free radical-induced protein damage in blood samples, a content of carbonyl derivatives of protein oxidative modification (OMP) based on the spectrophotometric measurement of aldehydic and ketonic derivatives in the blood was performed. The rate of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives of amino acid reaction with 2,4-dinitrophenylhydrazine (DNFH) as described by Levine et al. (1990) and as modified by Dubinina et al. (1995). DNFH was used for determining the contents of carbonyl groups in soluble and insoluble proteins. Carbonyl groups were determined spectrophotometrically from the difference in absorbance at 370 nm (aldehyde derivatives, OMP_{370}) and 430 nm (ketonic derivatives, OMP_{430}).

Statistical analysis

The mean ± S.E.M. values were calculated for each group to determine the significance of the intergroup difference. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors test ($p > 0.05$). The significance of differences between the levels of oxidative stress biomarkers (significance level, $p < 0.05$) was examined using the Kruskal–Wallis test by ranks (Zar, 1999). All statistical calculation was performed on separate data from each individual with Statistica 13.3 software (TIBCO Software Inc., Krakow, Poland).

Results and discussion

Figure 2 presents the values of TBARS levels obtained by incubating blood samples collected from patients with T2DM in the presence of aqueous extracts derived from roots and stalks of CM collected from rural and urban agglomerations. The final concentrations of extracts in the blood samples were 5 $mg.mL^{-1}$, 2.5 $mg.mL^{-1}$, 1.25 $mg.mL^{-1}$, and 0.63 $mg.mL^{-1}$.

Analyzing the final dose of CM extracts at 5 $mg.mL^{-1}$, we observed a statistically significant increase in TBARS level by 18.7% ($p < 0.05$) for root extracts of CM collected from urban areas (52.97 ±5.14 $nmol.mL^{-1}$) and by 35.1% ($p < 0.05$) for stalk extracts of CM collected from urban areas (60.31 ±3.77 $nmol.mL^{-1}$) compared to the untreated control samples (44.63 ±0.91 $nmol.mL^{-1}$). We obtained similar results after *in vitro* incubation of the blood samples with stalk extracts of CM collected from rural agglomerations at a dose of 5 $mg.mL^{-1}$, where we also recorded a statistically significant increase in TBARS levels by 17.2% ($p < 0.05$) compared to the control samples (52.31 ±3.98 $nmol.mL^{-1}$ vs. 44.63 ±0.91 $nmol.mL^{-1}$). We noted different trends after *in vitro* incubation of blood samples with root extracts of CM (at a final dose of 5 $mg.mL^{-1}$) collected from rural areas, where there was a non-statistically significant reduction (by 10%, $p > 0.05$) in the concentration of TBARS compared to the control samples (40.15 ±4.42 $nmol.mL^{-1}$ vs. 44.63 ±0.91 $nmol.mL^{-1}$).

By lowering the final dose of extracts to 2.5 $mg.mL^{-1}$, we observed the highest statistically significant increase in TBARS levels by 73.5% ($p < 0.05$) compared to the control samples using extracts derived from the roots of CM collected from urban areas (77.44 ±3.98 $nmol.mL^{-1}$ vs. 44.63 ±0.91 $nmol.mL^{-1}$). Using stalk extracts of CM

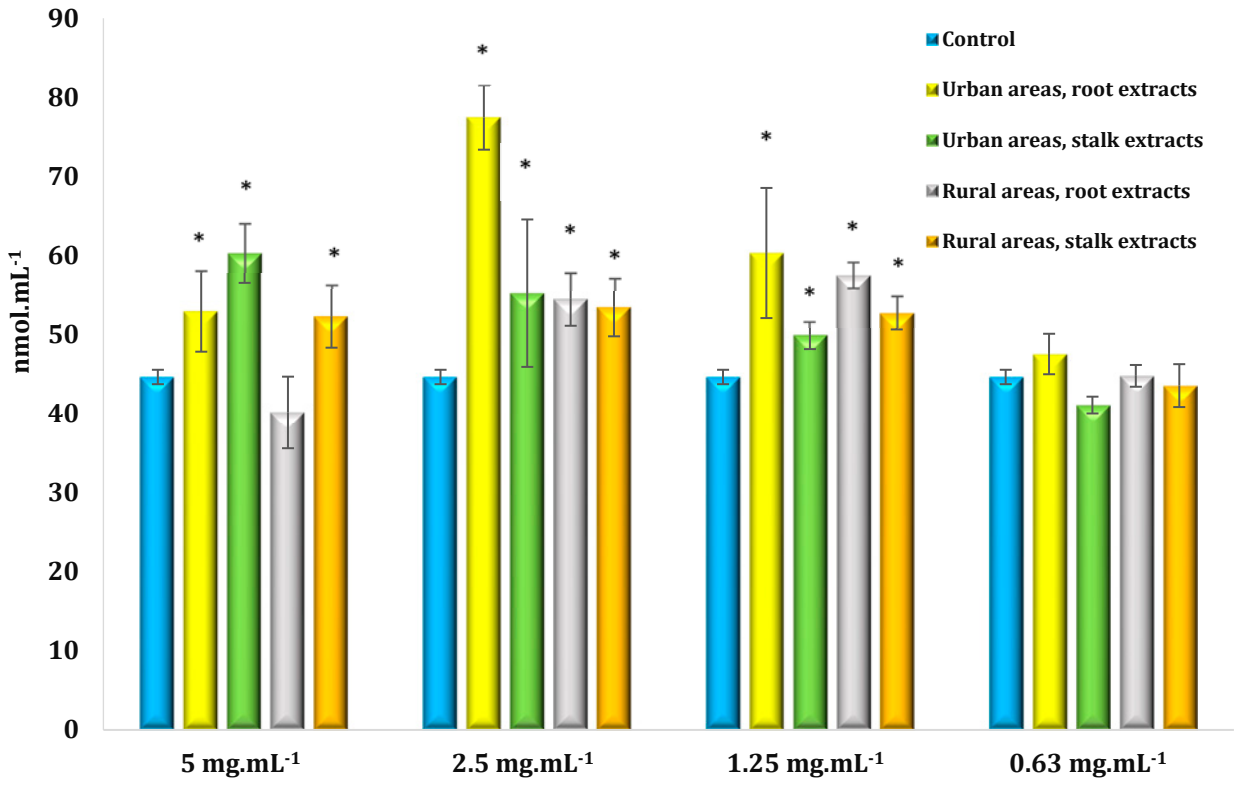

Figure 2 The TBARS content (nmol.mL^{-1}) as a biomarker of lipid peroxidation in the blood samples obtained from patients with type 2 diabetes mellitus after *in vitro* incubation with root and stalk extracts derived from *Chelidonium majus* L. collected from rural and urban areas of the Pomeranian region (M ±m, n = 8). The final concentrations of extracts in the blood samples were 5 mg.mL^{-1}, 2.5 mg.mL^{-1}, 1.25 mg.mL^{-1}, and 0.63 mg.mL^{-1}
*– statistically significant differences ($p < 0.05$) compared to the control samples

collected from urban areas (at a final dose of 2.5 mg.mL^{-1}) also resulted in a statistically significant increase in TBARS content by 23.8% ($p < 0.05$) compared to the control samples (55.23 ±9.23 nmol.mL^{-1} vs. 44.63 ±0.91 nmol.mL^{-1}). Similar results were obtained after incubating blood samples with extracts (at a final dose of 2.5 mg.mL^{-1}) derived from both roots (54.51 ±3.39 nmol.mL^{-1}) and stalks (53.49 ±3.72 nmol.mL^{-1}) of CM harvested from rural areas, where there was also a statistically significant increase in TBARS levels (by 22.1%, $p < 0.05$ and 19.9%, $p < 0.05$, respectively) compared to the control samples (44.63 ±0.91 nmol.mL^{-1}).

Similar results were obtained after lowering the final dose of the extracts used *in vitro* to 1.25 mg.mL^{-1}, where we also recorded a statistically significant increase in TBARS levels in the blood samples of diabetic subjects by 35.1% ($p < 0.05$) for root extracts of CM collected from urban areas (60.31 ±8.23 nmol.mL^{-1}) and by 11.9% ($p < 0.05$) for stalk extracts of CM collected from urban areas (49.95 ±1.79 nmol.mL^{-1}) compared to the control samples (44.63 ±0.91 nmol.mL^{-1}). After incubating the blood samples with extracts (at a final dose of 1.25 mg.mL^{-1}) derived from both roots (57.59 ±1.6 nmol.mL^{-1}) and stalks (52.82 ±2.17 nmol.mL^{-1}) of CM harvested from rural areas, we recorded statistically significant increases in TBARS levels by 29% ($p < 0.05$) and 18.4% ($p < 0.05$), respectively.

Similar but statistically no-significant increases in TBARS levels were obtained after incubating *in vitro* the blood samples with extracts (at a final dose of 0.63 mg.mL^{-1}) derived from roots of CM collected from both urban (47.54 ±2.56 nmol.mL^{-1} vs. 44.77 ±1.38 nmol.mL^{-1}) and rural areas (by 6.5%, $p > 0.05$ and 0.3% $p > 0.05$, respectively), compared to control samples. We noted different trends after using stalk extracts (at a final dose of 0.63 mg.mL^{-1}) of CM collected from urban (41.08 ±1.06 nmol.mL^{-1}) and rural (43.54 ±2.71 nmol.mL^{-1}) areas, where we recorded a reduction in TBARS levels compared to the control samples (44.63 ±0.91 nmol.mL^{-1}) (by 8% $p > 0.05$ and 2.4%, $p > 0.05$, respectively) (Figure 2).

The aldehydic and ketonic derivatives of oxidatively modified proteins in the blood samples of patients with T2DM after *in vitro* incubation with root and stalk

extracts derived from CM collected from rural and urban areas of the Pomeranian region were present in Figure 3.

Analyzing the results of levels of protein oxidation, we observed a statistically significant increase in the concentration of aldehydic derivatives of OMP after incubating the blood samples with extracts (at a final dose of 5 mg.mL^{-1}) derived from both roots (17.67 ±2.05 nmol.mL^{-1}) and stems (17.6 ±1.26 nmol.mL^{-1}) of CM collected from an urban agglomeration compared to the control samples (14.68 ±0.4 nmol.mL^{-1}). There was a statistically significant increase in levels of aldehydic derivatives of OMP by 20% ($p < 0.05$) and 19.9% ($p < 0.05$), respectively. We obtained similar results after *in vitro* incubation of the blood samples with root extracts of CM collected from rural areas, where we also observed a statistically significant increase in the levels of aldehydic derivatives of OMP by 56% ($p < 0.05$) compared to the control samples

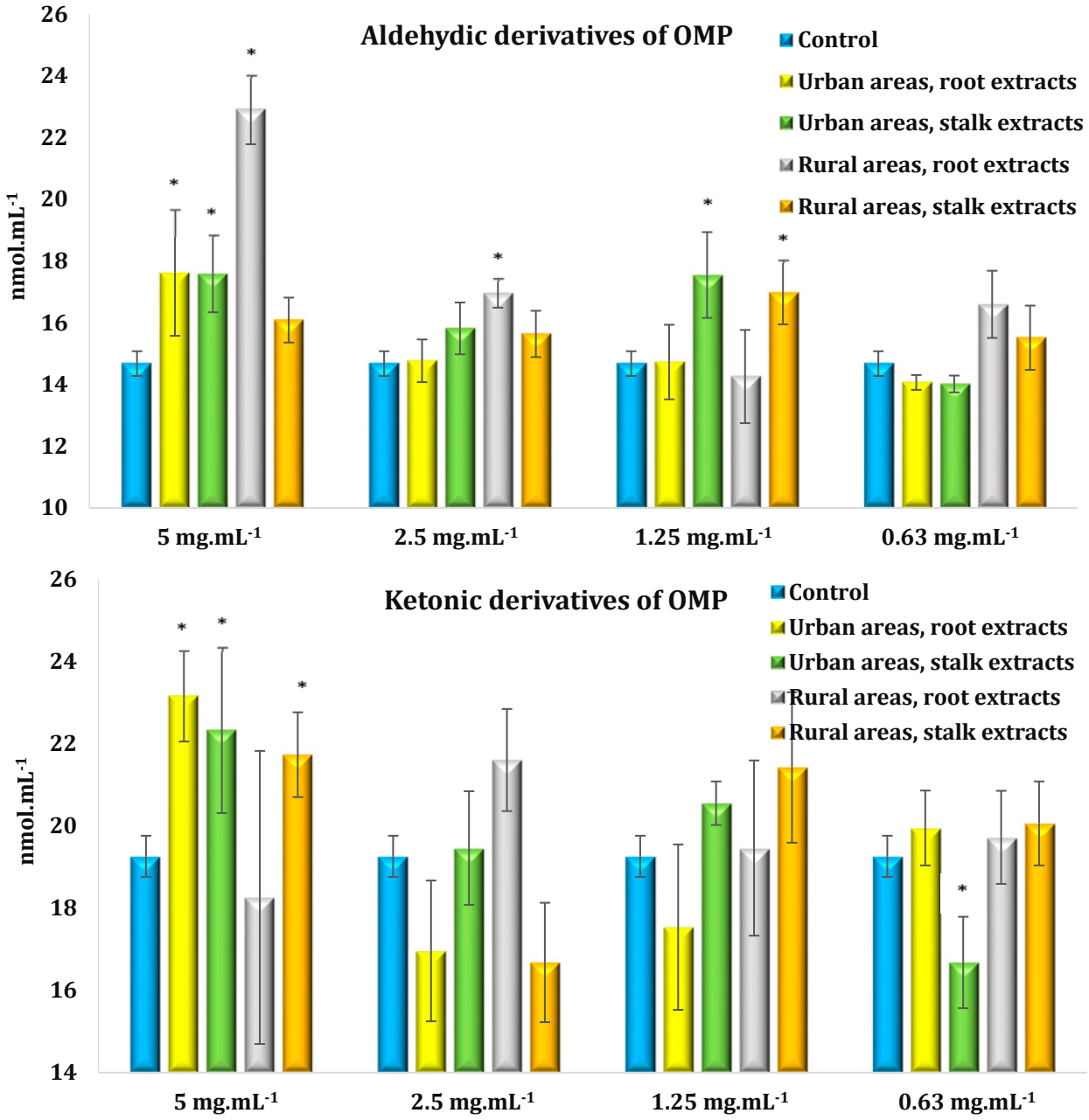

Figure 3 The aldehydic and ketonic derivatives of oxidatively modified proteins (nmol.mL^{-1}) in the blood samples of patients with type 2 diabetes mellitus after *in vitro* incubation with root and stalk extracts derived from *Chelidonium majus* L. collected from rural and urban areas of Pomeranian region (M ±m, n = 8). The final concentrations of extracts in the blood samples were 5 mg.mL^{-1}, 2.5 mg.mL^{-1}, 1.25 mg.mL^{-1}, and 0.63 mg.mL^{-1}
*– statistically significant differences ($p < 0.05$) compared to the control samples

(22.92 ±1.13 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1}). On the other hand, no statistically significant increase in the levels of aldehydic derivatives of OMP (by 9.6%, p >0.05) was observed after incubating the blood samples with stalk extracts of CM collected from rural areas compared to the control samples (16.09 ±0.73 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1}).

By lowering the dose of both root and stalk extracts from CM collected from urban areas to a value of 2.5 mg.mL^{-1} and incubating them with the blood samples, we also observed an increase in the level of aldehydic derivatives of oxidatively modified proteins compared to the control (14.77 ±0.69 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1} for root extracts; 15.82 ±0.84 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1} for stalk extracts). There was a statistically insignificant increase of 0.6% (p >0.05) and 7.8% (p >0.05), respectively. *In vitro* application of stalk extracts of CM collected from rural agglomerations to blood samples also resulted in a 6.5% (p >0.05) increase in levels of aldehydic derivatives compared to the control samples (15.64 ±0.75 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1}). Only incubation of root extracts of CM collected from rural areas (at a final dose of 2.5 mg.mL^{-1}) with blood samples resulted in a statistically significant elevation in levels of aldehydic derivatives of OMP by 15.6% (p <0.05) compared to the control samples (16.97 ±0.48 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1}).

We observed a statistically significant elevation in levels of aldehydic derivatives of OMP after *in vitro* incubation with stalk extracts of CM collected from both urban and rural areas with the blood samples (at a final dose of 1.25 mg.mL^{-1}) compared to the control samples (17.75 ±1.39 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1} after using stalk extracts of CM collected from urban areas; 17 ±1.05 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1} after using stalk extracts of CM collected from rural areas). This increase was by 19.6% (p <0.05) and 15.8% (p <0.05), respectively. After incubating the blood samples with root extracts of CM collected from urban areas (at a final dose of 1.25 mg.mL^{-1}), we observed a statistically no-significant increase in levels of aldehydic derivatives of OMP by 0.3% (p >0.05) compared to the control samples (14.73 ±1.21 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1}). We observed different trends after *in vitro* incubation of blood samples with root extracts of CM collected from rural areas (at a final dose of 1.25 mg.mL^{-1}), where there was a statistically no-significant decrease in levels of aldehydic derivatives of OMP by 2.9% (p >0.05) compared to the control samples (14.26 ±1.51 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1}).

The application of CM extracts at a final dose of 0.63 mg.mL^{-1} resulted in statistically no-significant changes in levels of aldehydic derivatives of OMP compared to the untreated control samples. We observed a decrease in levels of aldehydic derivatives of OMP after *in vitro* incubation of blood samples with extracts derived from both roots and stems of CM harvested from urban areas compared to the control samples (14.07 ±0.24 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1} for root extracts; 14.02 ±0.27 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1} for stalk extracts). This decrease was 4.2% (p >0.05) and 4.5% (p >0.05), respectively. Other results were obtained after incubation of blood samples with extracts derived from both roots and stalks of CM collected from rural agglomerations, where there was an increase in levels of aldehydic derivatives of OMP (by 13.1%, p >0.05 and 5.7%, p >0.05) compared to the control samples (16.6 ±1.09 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1} for root extracts; 15.52 ±1.04 nmol.mL^{-1} vs 14.68 ±0.4 nmol.mL^{-1} for stalk extracts) (Figure 3).

We observed a statistically significant increase in levels of ketonic derivatives of OMP after *in vitro* incubation with the blood samples of diabetic patients with extracts (at a final dose of 5 mg.mL^{-1}) derived from both roots and stalks of CM harvested from urban agglomerations compared to the control samples (23.16 ±1.11 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1} for root extracts; 22.33 ±2.02 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1} for stalk extracts). There was a statistically significant increase of 20.2% (p <0.05) for root extracts and 15.9% (p <0.05) for stalk extracts, respectively. We obtained similar results after incubating blood samples with stalk extracts of CM collected from rural areas, where there was also a statistically significant increase in levels of ketonic derivatives of OMP by 12.8% (p <0.05) compared to the control samples (21.73 ±1.03 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1}). Other results were obtained after incubating blood samples with root extracts of CM collected from rural areas (at a final dose of 5 mg.mL^{-1}), where we noted a statistically no-significant decrease in levels of ketonic derivatives of OMP (by 5.2%, p <0.05) compared to the control samples (18.26 ±3.56 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1}).

After incubating the blood samples with root extracts of CM collected from urban areas and stalk extracts of CM collected from rural areas (at a final dose of 2.5 mg.mL^{-1}), we observed a statistically non-significant decrease in levels of ketonic derivatives of OMP compared to control

samples (16.96 ±1.71 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1} for root extracts of CM collected from urban areas; 16.68 ±1.45 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1} for stalk extracts of CM collected from rural areas). There was a statistically significant increase of 11.9% ($p < 0.05$) for root extracts of CM collected from urban areas and 13.4% ($p < 0.05$) for stalk extracts of CM collected from rural areas, respectively. We obtained different results when we applied stalk extracts (at a final dose of 2.5 mg.mL^{-1}) of CM collected from urban areas (19.46 ±1.38 nmol.mL^{-1}) and root extracts of CM collected from rural areas (21.6 ±1.24 nmol.mL^{-1}) after incubation with the blood samples, where there was a statistically no-significant increase in levels of ketonic derivatives of OMP compared to the control samples (19.26 ±0.5 nmol.mL^{-1}). This increase was 1% ($p > 0.05$) for stalk extracts of CM collected from urban areas and 12.1% ($p > 0.05$) for root extracts of CM collected from rural areas, respectively.

After *in vitro* incubation of blood samples with root extracts of CM collected from urban areas (at a final dose of 1.25 mg.mL^{-1}), we observed a statistically no-significant decrease in levels of ketonic derivatives of OMP by 8.9% ($p > 0.05$) compared to the control samples (17.54 ±2.1 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1}). Other results were obtained after incubating blood samples with stalk extracts of CM collected from urban areas, where there was a no-significant increase in levels of ketonic derivatives of OMP by 6.7% ($p > 0.05$) compared to the control samples (20.55 ±0.53 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1}). We recorded similar results after *in vitro* incubation of blood samples with extracts derived from both roots (19.46 ±2.13 nmol.mL^{-1}) and stalks (21.42 ±1.83 nmol.mL^{-1}) of CM collected from rural agglomerations (at a final dose of 1.25 mg.mL^{-1}), where there was a statistically no-significant increase in levels of ketonic derivatives of OMP compared to the control samples (19.46 ±1.38 nmol.mL^{-1}). This increase was 1% ($p > 0.05$) for root extracts and 11.2% ($p > 0.05$) for stalk extracts, respectively.

Only at a final dose of 0.63 mg.mL^{-1} after incubation of blood samples with stalk extracts of CM collected from urban areas we observed a statistically significant decrease in levels of ketonic derivatives of OMP by 13.4% ($p < 0.05$) compared to the control samples (19.95 ±0.91 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1}). We obtained different results after *in vitro* incubation of blood samples with root extracts of CM collected from urban areas, where there was a statistical no-significant increase in levels of ketonic derivatives of OMP by 3.6% ($p > 0.05$) compared to control samples (16.68 ±1.11 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1}). We observed similar trends after incubating blood samples with extracts derived from both roots and stalks of CM collected from rural agglomerations, where there was a statistically no-significant increase in levels of ketonic derivatives of OMP compared to the control samples (19.72 ±1.13 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1} for root extracts; 20.06 ±1.02 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1} for stalk extracts). This increase was 2.4% ($p > 0.05$) and 4.2% ($p > 0.05$), respectively (Figure 3).

The current study is the continuation of our investigation aimed to assess the antioxidant and antibacterial properties of root and stalk extracts of CM collected from rural and urban areas of Pomeranian regions. In our previous study (Stefanowski et al., 2021d, e) on muscle tissue of rainbow trout (*Oncorhynchus mykiss* Walbum), we also demonstrated the antioxidant activity of CM extracts. Our results showed that extracts of CM collected from both urban and rural areas statistically significantly reduced the level of aldehyde derivatives of OMB by 18.8% ($p < 0.05$). The analysis of the levels of ketonic derivatives of OMP showed that extracts of CM collected from both urban and rural areas statistically significantly decreased the level of ketonic derivatives of OMP by 20.6 and 21.5%, respectively (for urban areas), as well as 26.7 and 12.5% (for rural areas). Lower levels of lipid peroxidation were observed after incubation with stalk extracts, while those collected from rural areas showed the lowest result (by 11%). Root extracts of CM collected from urban and rural areas increased TBARS levels. Analysis of oxidatively modified protein levels in the blood of rainbow trout after *in vitro* incubation with root and stem extracts shows that extracts can inhibit the production of oxidative carbonyls by scavenging free radicals.

Also, in another of our studies (Stefanowski et al., 2021a, b) on equine plasma, we demonstrated the antioxidant activity of CM extracts. Our results demonstrated that statistically significant reductions in lipid peroxidation byproducts were noted after incubation with extracts derived from roots of CM collected from both urban (by 35%, $p < 0.05$) and rural (by 34%, $p < 0.05$) agglomerations compared to the control samples. Stem extracts derived from CM also reduced TBARS levels, but only extracts derived from CM were collected from the rural areas; a statistically significant decrease (by 21%, $p < 0.05$) was observed compared to the control samples. The lowest values in the content of the aldehydic derivatives of OMP were observed after incubation with extracts derived from roots of CM collected from both rural and urban areas. On the other hand, levels of ketonic derivatives

of OMP were significantly increased after incubation with extracts derived from stems of CM collected from both rural and urban areas compared to the control samples, in contrast to extracts derived from roots of CM collected from urban areas, where there was a statistically significant reduction in ketonic derivatives of OMP (by 15%, $p < 0.05$) compared to the control samples.

We also demonstrated the antioxidant properties of CM extracts in the blood model of clinically healthy subjects (Stefanowski et al., 2021c). Our results showed, that the level of total antioxidant capacity (TAC) was statistically significantly changed in the human blood incubated with extracts derived from the stalks of CM collected from rural agglomerations (49.67 ±1.88%) compared to the untreated samples (63.18 ±5.07%). We observed different results after incubation of extracts derived from roots of CM collected from rural areas with human blood (62.12 ±1.88%) compared with the control samples (63.18 ±5.07%). Noting the results after incubation of human blood with root and stalk extracts of CM collected from urban agglomerations, we observed a decrease in TAC levels (61.67 ±3.2% for stalk extracts; 56.07 ±4.06% for root extracts) compared with the control samples (63.18 ±5.07%).

Studies by other researchers have shown that CM cell cultures are rich in polyphenolic compounds and isoquinoline alkaloids with confirmed antimicrobial, antioxidant, and anti-inflammatory properties. Chelerythrine, a natural benzo-phenanthridine alkaloid of CM, inhibited inflammatory and pain reactions in several *in vivo* and cell models employed by Lanfeld et al. (1981). *In vivo*, i.p. administration of the alkaloid (1–5 $mg.kg^{-1}$) alleviated mouse ear oedema, rat paw oedema, and abdominal constriction (pain reaction). Also, the isolated peritoneal macrophages upon treatment with 0.0001–1 $\mu g.ml^{-1}$ chelerythrine had dose-dependently reduced prostaglandin E2 and cyclooxygenase-2 expression. Alkaloid fraction and sanguinarine were efficient against carrageenan-induced rat paw oedema but chelerythrine showed lower activity (Lanfeld et al., 1981). However, in the later study by Mikołajczak et al. (2015), was demonstrated that various fractions of water extract at relatively high doses of 200 $mg.kg^{-1}$ body weight failed to alleviate the inflammation in a similar model. The crude water extract treatment aggravated the paw inflammation. Conversely, the extracts containing mainly coptisine and chelidonine were effective in the hot plate test for antinociceptive properties that suggests a supramedullary way of action (Mikołajczak et al., 2015).

Chelidonine is an isoquinoline alkaloid and the main alkaloid of CM. It has been also reported to have anti-cancer properties in a variety of tumour systems. Chelidonine and CM alkaloid extract were shown to overcome drug resistance by inhibiting the expression of p-glycoprotein (MDR-1) and several enzymes of the cytochrome P450 system, involved in xenobiotic metabolism in leukaemia and colon cancer cells and the induction of caspase-dependent apoptosis. However, published results concerning the effectivity and cancer-selectivity of chelidonine are still controversial. However, until now CM alkaloids have not been tested as possible therapeutic agents in cell lines and corresponding non-malignant primary cells of the mucosa of the upper respiratory tract (El-Readi et al., 2013; Herrmann et al., 2018).

Chelidonine exhibits effects on the central nervous system similar to those of morphine but weaker, and spasmolytic effects on smooth muscles similar to those of papaverine but also weaker. It is also a spindle poison. Chelerythrine strongly irritates the skin and mucous membranes, benumbs the central nervous system, and acts as a local anaesthetic (Perez Gultierrez, 2011; Isolani et al., 2012). Sanguinarine exhibits a vasorelaxant effect and an inhibitory effect on smooth muscle contractions. It is also an inhibitor of acetylcholinesterase and 5-lipoxygenase and it presents antimicrobial activity (Jagiełło-Wójtowicz et al., 1989; Miao et al., 2011). Protopine can act as an analgesic and inhibits histamine H1 receptors and thrombocyte aggregation. Coptisine was found to present neuroprotective, cytotoxic, and inhibitory effects on monoamine oxidase, and cardioprotective bioactivities (Wang et al., 2013).

In a study by Jang et al. (2021), it was found that chelidonine inhibited the proliferation of BxPC-3 and MIA PaCa-2 human pancreatic cancer cells in a dose- and time-dependent manner, confirming its apoptotic potential. In addition, flow cytometry analysis revealed that over 50% of BxPC-3 and MIA PaCa-2 cells exhibit early- and late-phase apoptosis after exposure to chelidonine (1 μM) for 24 h. These changes in expression levels following chelidonine treatment were re-confirmed through the analysis of transcription factor activity in both pancreatic cancer cell lines (Jang et al., 2021).

Nawrot et al. (2021) described the isolation and identification of a novel major latex protein (CmMLP1) composed of 147 amino acids and present a model of its structure containing a conserved hydrophobic cavity with high affinity to berberine, 8-hydroxycheleritrine,

and dihydroberberine. CmMLP1 and the accompanying three alkaloids were present in the eluted chromatographic fractions of latex. They decreased *in vitro* viability of human cervical cancer cells (HPV-negative and HPV-positive). The authors combined, for the first time, research on macromolecular and low-molecular-weight compounds of latex-bearing plants in contrast to other studies that investigated proteins and alkaloids separately. The observed interaction between latex protein and alkaloids may influence our knowledge of plant defense (Nawrot et al., 2021).

In the study of Noureini et al. (2017), the authors focused on the mechanism of telomerase inhibition by stabilization of telomeric G-quadruplex structures by berberine, chelerythrine, chelidonine, sanguinarine, and papaverine. Authors estimated telomerase activity and mRNA levels of hTERT using quantitative telomere repeat amplification protocol (q-TRAP) and qPCR, in MCF-7 cells treated with different groups of alkaloids. The results highlight the strong inhibitory effects of chelerythrine, sanguinarine, and berberine on telomerase activity, most likely through substrate sequestration. These isoquinoline alkaloids interacted strongly with the telomeric sequence G-quadruplex. In comparison, chelidonine and papaverine had no significant interaction with the telomeric quadruplex, while they strongly inhibited telomerase at the transcription level of hTERT (Noureini et al., 2017).

Sanguinarine (SNG), a natural compound of the Papaveraceae family, possesses favorable therapeutic potential against a variety of cancers. Prabhu et al. (2021) examined the underlying molecular mechanisms of SNG in non-small cell lung cancer (NSCLC) cells. SNG suppressed cell growth and induced apoptosis *via* downregulation of the constitutively active JAK/STAT pathway in all the NSCLC cell lines. siRNA silencing of STAT3 in NSCLC cells further confirmed the involvement of the JAK/STAT signaling cascade. SNG treatment increased Bax/Bcl-2 ratio, which contributed to a leaky mitochondrial membrane leading to cytochrome c release accompanied by caspase activation. In addition, the authors established the antitumor effects of SNG through reactive oxygen species (ROS) production, as inhibiting ROS production prevented the apoptosis-inducing potential of SNG. An *in vivo* tumor xenograft model further confirmed they're *in vitro* results. The study of Prabhu et al. (2021) investigated the molecular mechanisms by which SNG induces apoptosis in NSCLC, providing avenues for developing novel natural compound-based cancer therapies.

Conclusions

In the current study, we investigated the *in vitro* effects of CM extracts on lipid peroxidation and biomarkers of oxidatively modified proteins in the blood of patients with T2DM. The use of extracts derived from both roots and stalks of CM collected from both urban and rural agglomerations at final doses of 5.0 $mg.mL^{-1}$, 2.5 $mg.mL^{-1}$, and 1.25 $mg.mL^{-1}$ resulted in a significant enhancement of lipid peroxidation in the blood samples. On the contrary, only incubation of blood samples with extracts of stalks of CM collected from urban areas at a final dose of 0.63 $mg.mL^{-1}$ resulted in a no-significant decrease in TBARS level contributing to the protection of lipid structures in the membranes. Similar results were obtained by analyzing levels of aldehydic derivatives of oxidatively modified proteins in the blood of patients with T2DM after *in vitro* incubation with the extracts, where doses of 5.0 $mg.mL^{-1}$, 2.5 $mg.mL^{-1}$, and 1.25 $mg.mL^{-1}$ significantly increased the oxidation process of protein structures. Analysis of levels of ketonic derivatives of oxidatively modified proteins showed that the use of root extracts of CM collected from urban agglomeration at final doses of 2.5 $mg.mL^{-1}$ and 1.25 $mg.mL^{-1}$ reduced levels of ketonic derivatives, while the use of stalk extracts of CM harvested in urban agglomerations at a final dose of 0.63 $mg.mL^{-1}$ statistically significantly reduced levels of ketonic derivatives of oxidatively modified proteins compared to the control samples. The comparison of these results showed that CM extracts can effectively inhibit the formation of protein carbonyls by the elimination of free radicals. This phenomenon may explain the use of CM in medicine through its destructive effect on the membrane structures of cancer cells, due to the presence of a wide range of active compounds and other secondary metabolites throughout the plant. Our results may suggest that CM is a rich source of biomolecules that exhibit cytotoxic properties.

Conflict of interests

The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

Acknowledgements

This research was supported by the Pomeranian University in Słupsk (Poland).

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

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Characteristic of *Salvia officinalis* L. genotypes in the Steppe of South Ukraine

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Article Details:
Received:2022-07-20
Accepted:2022-08-11
Available online:2022-11-30

DOI: https://doi.org/10.15414/ainhlq.2022.0021

In this study gives the characteristic of *Salvia officinalis* L. genotypes (Lamiaceae)grown in the Southern Steppe of Ukraine. *S. officinalis* one of the most well-known plants from *Salvia* L. genus that is used as an aromatic, medicinal, and culinary herb. The plant raw material of this plant is characterized by numerous biological activities that allow use in the pharmacological industry. This plant is characterized by morphological polymorphism and the selection of new genotypes within *S. officinalis* has a polyfunctional meaning. The plant material of this study was the genotypes of *S. officinalis* (108-14, 109-14, 108-14-1, 108-14-2, and 113-16). It investigated 3d years of living plants at the flowering stage from the experimental collections of aromatic and medicinal plants in the Kherson region, v. Plodove (Institute of Climate Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine). The height of plants of investigated genotypes was 65.02–86.37 cm, the diameter of the plant was 89.37–117.03 cm, the leaf length was 8.20–11.06 cm, the leaf width was 2.95–3.79 cm, the inflorescence length was 14.52–26.01 cm, the inflorescence diameter was 4.07–5.57 cm, the number of whorls in inflorescences was 8.12–10.55, and the number of flowers in the whorl was 10.02–12.14. The color of the leaf surface, the character of the leaf surface (wrinkles), and colour of the flowers depended on genotype. The mass of raw from the shrub was determined from 350.11 to 560.27 g and the average mass of essential oil of one plant was from 1.37 to 2.01 g depending on genotype. The essential oil content in the herb, leaves, and inflorescences on dry mass was 1.0–1.5%, 1.09–1.79%, and 0.98–1.28%, respectively. In the herbs and leaves, minimal and maximal content of oil were found in genotypes 113–16 and 109–14, respectively. These results can be useful for further selective work within *S. officinalis* species and including other species of this genus.

Keywords: dalmatian sage, morphometric features, essential oil

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Introduction

The study of plants of the *Salvia* L. genus is still actual due to polyfunctional use in human life. *Salvia* species are widely diverse and used as a culinary herb, spice, medicinal, cosmetic, and ornamental plants in many countries in the world (Hamidpour et al., 2014; Grdiša et al., 2015; Sharopov et al., 2018).

Salvia genus is one of the most groups of Lamiaceae Martinov and includes approximately 1000 species which are distributed in Eurasia, Africa, and America (González-Gallegos et al., 2020). Some species are cultivated widely and among them, *Salvia officinalis* L. (dalmatian sage) is the most well-known and used (Hassiotis, 2018). The ancient Romans cultivated this popular culture as an ornamental and honey-bearing plant. Its homeland is considered to be Asia Minor, from where it spread across the Balkan Peninsula and the Mediterranean (El Euch et al., 2019).

The essential oil of sage leaves contains tannins, ursulic and oleic acids, alkaloids, and flavonoids. The main action of clary sage is antiseptic and anti-inflammatory (Sharopov et al., 2018). The plant also has astringent, emollient, and hemostatic properties, and also reduces sweating. It promotes the secretion of gastric juice and has mild antispasmodic properties (Vosoughi et al., 2018; Hudz et al., 2020). In this regard, the infusion of leaves is used to treat such diseases of the digestive system as gastritis, peptic ulcer disease of the stomach and duodenum with reduced acidity of gastric juice, and spastic colitis. Infusion of leaves is also used for catarrh of the upper respiratory tract, chronic bronchitis, angina, and inflammatory processes of the oral cavity and pharynx (El Eough et al., 2019). Some foreign scientists emphasize the positive effect of sage extracts on human cognitive activity (Wightman et al., 2021). The essential oil demonstrated antibacterial, antifungal (Agrawal et al., 2021), and antiviral (Rashidipour et al., 2022) activities. Due to this fact, sage is suitable for the biological protection of agricultural plants from fungal pathogens, and is also a good repellent (Vosoughi et al., 2018; Harizia et al., 2021; Khaled-Gasmi et al., 2021; Morkeliūnė et al., 2021). The chemical composition of the essential oil of *S. officinalis* can explain the allelopathic potential of this plant (Bouajaj et al., 2013).

The results of these studies suggest the use of the essential oil of both species as an effective natural anti-inflammatory and antiviral agent (Abou Baker et al., 2021). The study of the antioxidant properties, including medicinal sage, grown in the South of Italy, concluded the possibility of using them in functional food products, herbal medicines, or as a source of active biomolecules (Vergine et al., 2019). The well-deserved attention to clary sage is justified by the use of its essential oil as a food preservative and auxiliary agent in bacterial food toxic infections (Selim et al., 2022).

According to literature data, the species *Salvia officinalis* is characterized by a great polymorphism of some morphological and biochemical parameters, which allows carry out the individual selection (Jug-Dujaković et al., 2012; Shakoor et al., 2021). Considering the significance of useful properties of *S. officinalis* raw in human life, it's important to find and select new features of plants during the period of vegetation.

Our work aimed to study some morphological and economically valuable features such as the yield and mass fraction of essential oil in the hybrid forms of *Salvia officinalis* in the conditions of the Steppe zone of Southern Ukraine for further selective work.

Material and methodology

Biological material

The research material was the genotypes of *Salvia officinalis* L. (108-14, 109-14, 108-14-1, 108-14-2, and 113-16) (Figure 1a, b). The research was conducted in the Institute of Climate Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine (experimental collections of aromatic and medicinal plants in the Kherson region, v. Plodove). It investigated 3-year-old plants at the flowering stage during 2020–2021.

Morphometric parameter study and morphological describing

The morphometric parameters of plants were used in this study: height of the plant (in cm), the diameter of the plant (in cm), length and width of leaves (cm), length and diameter of inflorescence (in cm), the number of whorls in the inflorescences, the number of flowers in the whorls. Also, were described leaf surface (colour and wrinkle), and inflorescences (colour).

The essential oil content determination

The essential oil was obtained from the herb (aerial mass of plants), leaves, and inflorescences at the flowering stage; the mass fraction of the essential oil was determined by the hydrodistillation method (Ginsberg's method) on the Clevenger apparatus, based on the absolutely dry mass of the plant material (Elyemni et al., 2019).

Figure 1a Selected genotypes of *Salvia officinalis* L. at the flowering stage with inflorescences
A – 108-14; B – 108-14-1

Figure 1b Selected genotypes of *Salvia officinalis* L. at the flowering stage with inflorescences
C – 108-14-2; D – 109 -14; E – 113-16

Statistical analysis

The results are expressed as mean values of three replications ± standard deviation (SD); hierarchical cluster analyses of similarity between samples were computed based on the Euclidean similarity index. Data were analyzed with the ANOVA test and differences between means were compared through the Tukey-Kramer test ($p < 0.05$).

Results and discussion

The conditions of the Kherson region promote the cultivation of promising aromatic plants. Especially those species that can be grown in conditions of insufficient soil and air moisture in the South of Ukraine (Svydenko and Yezhov, 2014; Dudchenko et al., 2020). The most common among them are lavender, lavandin (Fernández-Sestelo and Carriello, 2020; Pokajewicz et al., 2021), lemon wormwood (Korablova et al., 2020), medicinal hyssop, peppermint (Yezerska et al., 2021), thyme (Vergun et al., 2022), monarda (Dudchenko et al., 2020), sage (Korablova et al., 2019), the raw materials of which are used in the pharmaceutical, perfumery and cosmetic, food industry, and medicine (Mňahončaková et al., 2019; Hudz et al., 2020; Frolova et al, 2021; Korablova et al., 2021).

In the first year of life, all *S. officinalis* plants vegetated and no special differences were observed between them except for the color and size of the leaf blade. The vegetation of plants in the second year was noted in April, the budding stage occurred in May, and the

Table 1 Characteristics of genotypes of *Salvia officinalis* L. according to morphometric indicators and signs at the flowering stage

Parameter	Genotype				
	108-14	108-14-1	108-14-2	109-14	113-16
Height of plant, cm	72.21 ±4.02^{ab}	67.19 ±2.61^{b}	66.94 ±3.21^{b}	86.37 ±3.41^{a}	65.02 ±2.90^{b}
Diameter of plant, cm	115.94 ±6.01^{a}	103.04 ±4.81^{b}	100.18 ±5.11^{b}	117.03 ±5.12^{a}	89.37 ±6.71^{c}
Length of leaf, cm	11.06 ±1.56^{a}	9.96 ±0.14^{b}	9.51 ±0.81^{b}	10.09 ±1.10^{a}	8.20 ±2.11^{b}
Width of leaf, cm	3.79 ±0.22^{a}	2.95 ±0.18^{b}	3.51 ±0.30^{a}	3.56 ±0.21^{a}	3.22 ±0.31^{a}
Colour of the leaf surface	green with grey	light-green with grey	light green	dark green	grey
The character of leaf wrinkle	moderate	moderate	strong	strong	moderate
Length of inflorescence, cm	22.02 ±1.15^{b}	21.02 ±1.54^{b}	26.01 ±1.54^{a}	17.35 ±1.51^{c}	14.52 ±0.51^{d}
Diameter of inflorescence, cm	4.07 ±0.34^{c}	5.54 ±0.31^{a}	5.57 ±0.50^{a}	4.53 ±0.31^{c}	5.08 ±0.41^{b}
The number of whorls in the inflorescences	10.55 ±0.23^{a}	8.12 ±0.18^{c}	9.03 ±0.11^{b}	8.45 ±0.16^{c}	8.23 ±0.52^{c}
The number of flowers in the whorls	11.23 ±0.42^{ab}	12.14 ±0.63^{a}	10.02 ±0.08^{b}	10.28 ±0.21^{b}	10.66 ±0.87^{b}
Color of inflorescence	light purple	rose	light blue	purple	white

Note: within a row, means without a common superscript differ ($p < 0.05$)

beginning of flowering registered in the third decade of May. The period of mass flowering was observed in June and the fruiting period in July (Lichinkina and Svydenko, 2006).

Plants of investigated genotypes at the flowering period occur almost at the same time with a difference of 1–2 days. Genotype 113-16 was flowered first (18.05), and genotype 109-14 was flowered last (21.05). Genotypes 109-14 and 108-14 stood out among the samples of medicinal sage according to the height of the shrub. Genotype 113-16, which differs from others in the white color of the flower, had the lowest shrub height.

The study of complex features of plants among which morphometrical and morphological peculiarities are an important aspect of the assessment of selective work to highlight new genotypes (Çamlica and Yaldiz, 2019; Vergun et al., 2021).

The morphological and morphometrical characteristics of selected genotypes are represented in Table 1. The height of selected *S. officinalis* genotypes varied from 66.94 to 86.37 cm. The diameter of investigated plants achieved 89.37–117.03 cm. The length and width of leaves were 8.20–11.06 and 2.95–3.79 cm, respectively. We also found differences in the colour of the leaf surface and inflorescences for all genotypes and the character of the leaf wrinkle for 108-14-2 and 109-14 genotypes where this parameter was highlighted stronger. The length and diameter of inflorescences were 14.52–26.01 and 4.07–5.57 cm, respectively.

According to Mossi et al. (2011), S.officinalis plants from different origins and propagation forms had a height of plants 30.5–55.3 cm, a width of leaves of 1–3 cm, a length of leaves 2.5–9.0 cm, a length of inflorescences 11.0–20.5 cm.

It is generally known that the raw material of sage is the entire above-ground mass of plants. The yield of above-ground mass in plants in the third year of vegetation varied from 350 to 560 g. The highest productivity was genotype 109-14, and the smallest was genotype 113-16 (Table 2).

The essential oil in sage is accumulated in glandular scales, glandular hairs, and unicellular and multicellular essential oil glands (Kutko et al., 2002). The mass of essential oil from one plant was from 1.37 to 2.01 g.

Table 2 The yield of selected genotypes of *Salvia officinalis* L. at the end of flowering

Parameters	Genotype				
	108-14	108-14-1	108-14-2	109-14	113-16
The mass of raw from shrub, g	500.13 ±20.1^{ab}	420.09 ±19.2^{b}	480.45 ±23.3^{b}	560.27 ±30.1^{a}	350.11 ±16.7^{c}
The mass of essential oil from one plant, g	2.01 ±0.33^{a}	1.37 ±0.09^{c}	1.54 ±0.11^{b}	1.62 ±0.12^{b}	1.67 ±0.08^{b}

Note: within a row, means without a common superscript differ ($p < 0.05$)

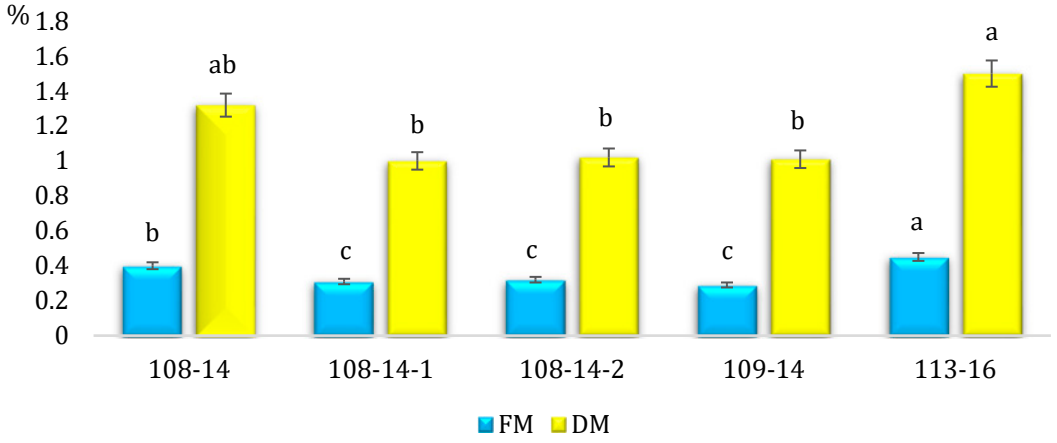

Figure 2 The mass fraction of essential oil of herb of *Salvia officinalis* L. genotypes at the end of flowering
FM – % of fresh mass; DM – % of dry mass. Different superscripts in each column indicate the significant differences in the mean at p <0.05

According to Lichinkina and Svydenko (2006), the yield of leaves from one plant of *S. officinalis* in the Kherson region was 240 g, and the mass of the shrub was 430 g. The fresh weight of conventionally grown plants was 271 g and hydroponically grown 321 g (Traykova et al., 2019).

A significant variation in yield and composition was found among and within *Salvia* species that depend on the origin of raw (Rajabi et al., 2014). Also, some results proved that the use of growth regulators significantly affects essential oil content and components of *Salvia* raw (Rowsan et al., 2010). The period of growth along with other peculiarities is a very important sign, for example, after collection of Turkish *Salvia aramiensis* at the pre-flowering stage, flowering, and post-flowering stages were determined 2.2, 1.0, and 2.1% of essential oil (Demirci et al., 2002). In another study, *S. officinalis* essential oil content was highest during the flowering period (Farhat et al., 2016). It is known that the content of essential oil in clary sage plants depends on the agro-technique of cultivation, season, and the genotype (Pitarević et al., 1984; Vosoughi et al., 2018; Hazrati et al., 2022). All organs of clary sage (*S. sclarea*) are covered with trichomes, but simple and capitate hairs practically do not contain essential oil (Kutko et al., 2002; Svydenko and Lichinkina, 2005). The glandular trichomes of Lamiaceae have important functional and taxonomic meanings but their character differs significantly (Kahraman et al., 2010; Svydenko et al., 2018). The main amount of essential oil is concentrated in essential oil glands, which we also found on leaves, stems, calyxes, and petals.

According to our research, the mass fraction of essential oil in samples in the phase of the end of flowering

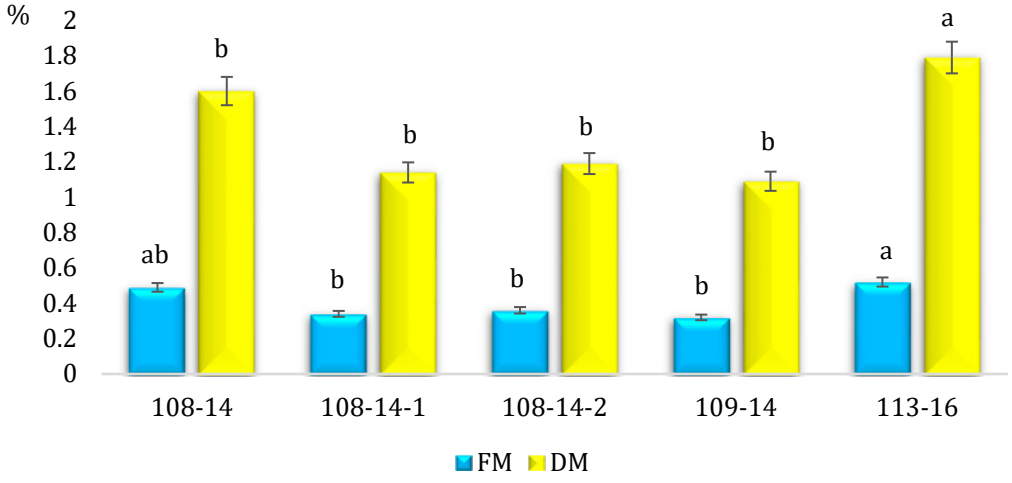

Figure 3 The mass fraction of essential oil of *Salvia officinalis* L. genotypes in the leaves at the end of flowering
FM – % of the fresh mass; DM – % of dry mass. Different superscripts in each column indicate the significant differences in the mean at p <0.05

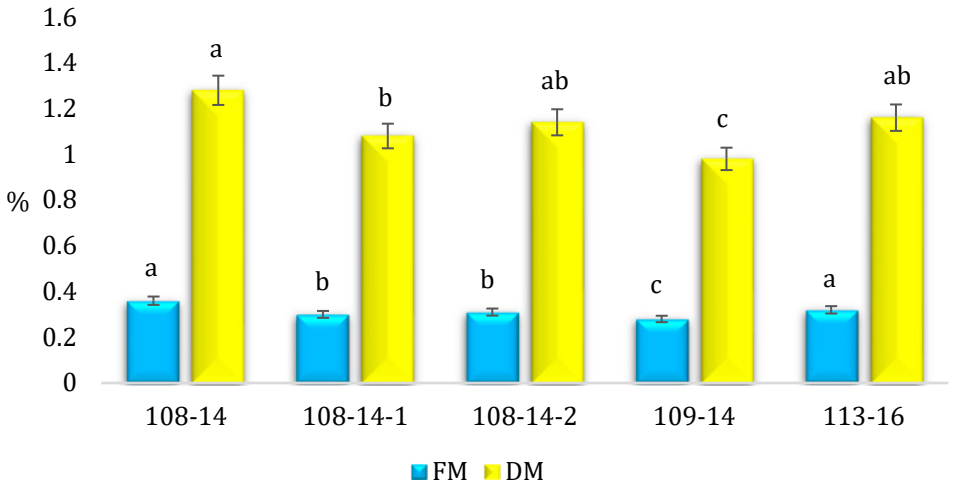

Figure 4 The mass fraction of essential oil of *Salvia officinalis* L. genotypes in the inflorescences at the end of flowering FM – % of the fresh mass; DM – % of dry mass. Different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

ranged from 0.29 to 0.45% of the fresh mass of plant material or from 1.0 to 1.5% of completely dry (Figure 2). We recorded the largest mass fraction of essential oil in genotype 113-16 and the smallest in genotype 109-14.

The content of *S. officinalis* essential oil from Poland was in May 1.16% and in August 1.35% (Zawiślak, 2014). The essential oil yield of *S. officinalis* fruits, according to Taarit et al. (2009), was 0.39%. As reported Verma et al. (2015), the essential oil yield for these plants from Southern India was 0.22–0.43% for the whole plant and 0.15–0.60% for different parts of the plant.

The content of essential oil depends on many factors among which part of the plant (Sellami et al., 2011). In this study, we determined essential oil mass in the leaves and inflorescences separately. It was determined the content of essential oil of 0.34–0.52% in fresh mass and 1.09–1.79% in dried mass (Figure 3). The highest content of essential oil is determined in leaf raw of the genotype 113-16 and the least in genotype 109-14.

According to Couladis et al. (2002), the interpopulation variation of essential oil content of leaves and flowers of *S. officinalis* from Serbia averaged 1.41 and 1.13%, respectively. The content of essential oil in inflorescences was 0.28–0.36% in fresh mass and 0.98–1.28% in dried mass (Figure 4). The minimal content of essential oil is found in inflorescences raw of genotype 109-14 and maximal in genotype 108-14.

Conclusions

Thus, in conditions of the South Step zone of Ukraine were selected five genotypes of *S. officinalis* and studied for selected morphometrical and morphological peculiarities. Studying the economically valuable features, we established that the mass of the raw per plant, the mass fraction of the essential oil, and the morphometrical parameters of plants depended on the genotypes. All plants differed by inflorescence and leaf surface colour, and character of surface (wrinkle). The minimal mass of the shrub, and essential oil content of the herb and leaves was detected for genotype 113-16. The maximal mass from the shrub, essential oil content of herbs and leaves were determined for genotype 109-14. However, the essential oil content of inflorescences for genotype 109-14 was minimal and for genotype 108-14 maximal. These results can be useful for further selective work within *S. officinalis* species and including other species of this genus. The creation of new varieties will be very useful for pharmaceutical, cosmetic, and food industries and as ornamental plants.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

Funding

This work was supported by grant Visegrad Fund and SAIA (Slovakian National Scholarship Program) for the scholarship for the research during which the presented knowledge was obtained.

Acknowledgements

The publication was prepared with the active participation of researchers in International Network AgroBio*Net*.

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

OPEN ACCESS

Monitoring of selected heavy metals and bioactive compounds in potato (*Solanum tuberosum* L.) tubers

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Article Details:
Received:2022-07-20
Accepted:2022-08-13
Available online:2022-11-30

DOI: https://doi.org/10.15414/ainhlq.2022.0022

Potato (*Solanum tuberosum* L.) tubers are a significant part of agricultural production and an important part of the food for human nutrition. Tubers of seven potato cultivars (Annalena, Anuscha, Elfe, Impala, Marena, Riviera, Rosara) were analyzed in this study. Total polyphenol content in analyzed cultivars ranged from 161.7 to 496.1 mg GAE.kg^{-1} FW (641.5 to 2418.6 mg GAE.kg^{-1} DM). Antioxidant activity in analyzed cultivars ranged from 0.27 to 0.67 mmol TE.kg^{-1} FW (1.08 to 6.18 mmol TE.kg^{-1} DM). Significant differences were determined in the TPC and AA of Annalena and Rosara, Anuscha and Rosara, Annalena and Riviera, and Anuscha and Riviera. Cd content in analyzed cultivars ranged from 0.006 to 0.064 mg.kg^{-1} FW (0.02 to 0.278 mg.kg^{-1} DM). Pb content in analyzed cultivars ranged from 0.015 to 0.370 mg.kg^{-1} FW (0.05 to 1.47 mg.kg^{-1} DM). The limit for Pb, set by Commission Regulation (EC) no. 1881/2006 was exceeded in cultivars Annalena, Elfe, Impala, Marena, and Rosara. Significant differences were determined in the Cd content of Annalena and Marena, Annalena and Riviera, and Annalena and Rosara. Significant differences were determined in the Pb content of Annalena and Riviera, and Annalena and Rosara. Positive correlations ($p < 0.001$) were determined between TPC and AA ($r = 0.658$). Negative correlations were determined ($p < 0.05$) between TPC and Cd content, and AA and Cd content.

Keywords: *Solanum tuberosum*, polyphenols, antioxidants, cadmium, lead

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Introduction

Potato (*Solanum tuberosum* L.) tubers are the third most important crop in the world (after rice and wheat) and they contribute to the nutrition of people in many parts of the world (Venkatasalam et al., 2019). According to FAOSTAT, worldwide potato consumption reached 234802223.6 tonnes in 2019 (30.66 kg per capita).

Potato tubers are primarily composed of carbohydrates, but they are also an important source of proteins and essential amino acids, fiber, potassium, and other minerals, vitamin C, B_6, and bioactive compounds (Pineros-Nino et al., 2017; Sood et al., 2017). Nutritional properties such as starch digestibility, glycemic index, and relative glycemic impact are important for human health. The relationship between potato tuber composition and its effect on blood glucose release has been explored through various *in vitro* and *in vivo* studies (Singh and Kaur, 2016).

Potatoes are known as one of the richest sources of antioxidants in the human diet (Ramadan and Oraby, 2016). Polyphenols are considered the most widespread antioxidants in the human diet, able to prevent the formation of free radicals with harmful effects on health, and are therefore important in reducing the risk of developing diseases (Amoroso et al., 2019). These compounds may have multiple effects such as reduction of the initiation of tumors, the induction of apoptosis and platelet aggregation, modulation of lipid metabolism, stimulation of the immune system, and antibacterial, antiangiogenic, and antimutagenic properties (Laib and Barkat, 2018). Studies on animals or cultured human cell lines demonstrate the role of polyphenols in the prevention of cardiovascular diseases, cancer, neurodegenerative diseases, osteoporosis, and diabetes (Scalbert et al., 2005). The content of phenols in potatoes is highly dependent on the cultivar, cultivation, stress factors, growing conditions, climatic conditions, tuber maturity level, and processing (Hamouz et al., 2006; Sood et al., 2017; Laib and Barkat, 2018).

Potato plants are exposed to different types of compounds that are present in polluted air, soil, and water. Foreign elements represent a stress factor for most plants, and after entering the food chain, they can pose a danger to the human organism. (Goncalves et al., 2009; Musilová et al., 2011). Heavy metals are involved in reducing the quality of the environment. Non-essential chemical elements, such as Cd and Pb are toxic even at relatively low concentrations (Musilová et al., 2017).

Cadmium (Cd) is an element with highly toxic effects on humans due to its cumulative effect, mainly in the liver and kidneys. Cadmium is naturally present in most soils as a trace element, but it is also widespread in the environment due to human activity (Mengist et al., 2017).

Lead (Pb) is a highly toxic element with effects on almost every organ in the body, with the most affected target being the nervous system. Major anthropogenic sources of Pb, such as smelting, lead-based painting, lead-containing pipes, and lead-acid batteries battery, etc. contribute to the adverse effects of lead on humans and the environment (Ara and Usmani, 2015).

This study aimed to investigate and evaluate the total content of polyphenols and antioxidant activity in selected cultivars of potato tubers, determine the presence of selected heavy metals in selected cultivars of potato tubers, and evaluate the correlations between heavy metal content and the total content of polyphenols and antioxidant activity.

Material and methodology

Plant samples

Samples of potato (*Solanum tuberosum* L.) cultivars (Annalena, Anuscha, Elfe, Impala, Marena, Riviera, Rosara) were taken at the stage of full maturity from selected localities of the Slovak Republic (Nedanovce, Bytčica, Spišská Stará Ves).

Extract preparation

25 g of homogenized potato tubers were extracted in 50 mL of 80% methanol for 12 hours and filtered through Munktell No. 392 filtrating paper.

Total polyphenol content

Total polyphenol content was determined by Folin-Ciocalteau colorimetric method (Lachman et al., 2003) using Folin – Ciocalteu phenol reagent (Merck, Germany), 20% Na_2CO_3 (Sigma Aldrich, USA), and distilled water. 0.1 mL of extract was pipetted into a 50 mL volumetric flask. 0.85 mL of Folin Ciocalteau reagent was added, and after 3 minutes, 5 mL of 20% Na_2CO_3 was added. After stirring the mixture, flasks were filled with distilled water to the mark. Flasks were left for 2 hours at laboratory temperature and then measured againsta blank solution at 765 nm, using Shimadzu UV/VIS scanning spectrophotometer. Total polyphenol content was expressed as mg of gallic acid equivalent in 1 kg of fresh matter, based on the calibration curve ($R^2 = 0.996$)

Antioxidant activity

Antioxidant activity was measured by DPPH radical scavenging assay (Brand Williams et al., 1995), using DPPH•+ radical (2,2-diphenyl-1-picrylhydrazyl) (Sigma Aldrich, USA). DPPH•+ radical and methanol (Sigma Aldrich, USA) were used to produce a working DPPH solution. 1 mL of extract was pipetted into 3.9 mL of working DPPH solution, stirred, and left in dark. After 10 minutes, the solution was measured against a blank solution at 515.6 nm, using Shimadzu UV/VIS scanning spectrophotometer. Antioxidant activity was expressed as mmol of Trolox equivalent in 1 kg of fresh matter, based on the calibration curve (R^2 = 0.997).

Cadmium and lead content

Cd and Pb content were determined after mineralization in a mixture of 5 mL of HNO3 (Suprapur®, Merck, Darmstadt, Germany) and 5 mL of deionized water (0.054 μS.cm^{-1}) in the Mars Xpress 5 closed microwave digestion system (CEM Corp., Matthews, NC, USA). Mineralized samples were analyzed by the atomic absorption spectrometer SpectrAA 240Z. The limit of detection of Cd and Pb was set at 10 ng.kg^{-1}, and the limit of quantification was set at 30 ng.kg^{-1}.

Statistical analysis

Statistical analysis was performed using XLSTAT software. To determine significant differences ($p < 0.001$) between individual cultivars, Kruskal-Wallis nonparametric ANOVA test, and Dunn pairwise test were performed. The Spearman correlation coefficient was performed to determine significant correlations between monitored parameters.

Results and discussion

Total polyphenol content and antioxidant activity of potato tubers

Total polyphenol content in analyzed cultivars ranged from 161.7 to 496.1 mg GAE.kg^{-1} FW (641.5 to 2418.6 mg GAE.kg^{-1} DM). Significant differences ($p < 0.001$) were determined between the TPC of Annalena and Rosara, Anuscha and Rosara, Annalena and Riviera, and Anuscha and Riviera. The difference in the content of polyphenols can be attributed to the variety, genotype, and harvesting sites, which influence the accumulation of phenolic compounds (Hesam et al., 2012). André et al. (2009) reported that the content of total phenols in the tubers of 13 potato cultivars was significantly influenced by the growing environment. However, the genotypic component (cultivar) contributed the most to the observed variability. Giusti et al. (2014) reported the polyphenol content from 1133.7 to 1146.3 mg GAE.kg^{-1} DW in yellow-fleshed potatoes. Gugała et al. (2017) reported this parameter from 161.9 to 179.2 mg GAE.kg^{-1} FW in potato tubers. Lachman et al. (2008) reported TPC from 2904 to 3295 mg GAE.kg^{-1} DM, depending on the cultivar and location. Franková et al. (2022) reported about wide range of values from 1140 to 33400 mg GAE.kg^{-1} DM, depending on cultivar and maturity. Deußer et al. (2012) also reported about a wide range of values from 400 to 5400 mg GAE.kg^{-1} DM in potatoes grown in Luxembourg. Karim et al. (2017) reported about higher values of polyphenol content from 3205.9 to 5289.4 mg GAE.kg^{-1} DM. The cultivar significantly influenced the TPC content. Hamouz et al. (2007) also reported higher values – from 3040 to 7700 mg GAE.kg^{-1} DM depending on the cultivar, location, and fertilization. Hesam et al. (2012), however, reported about lower values of TPC from 165.7 to 362.4 mg GAE. kg^{-1} DM.

Antioxidant activity of analyzed cultivars ranged from 0.27 to 0.67 mmol TE.kg^{-1} FW (1.08 to 6.18 mmol TE.kg^{-1} DM). Significant differences ($p < 0.001$) were determined between AA of Annalena and Rosara, Anuscha and Rosara, Annalena and Riviera, and Anuscha and Riviera. Kita et al. (2013) reported that AA was from 4.6 to 14.4 mmol TE.kg^{-1} DM in potato cultivars. According to Hu et al. (2012), several factors influence antioxidant activity, namely maturity,

Table 1 Total polyphenol content and antioxidant activity of analyzed potato cultivars

Cultivar	TPC (mg GAE.kg^{-1} FW)	TPC (mg GAE.kg^{-1} DM)	AA (mmol TE.kg^{-1} FW)	AA (mmol TE.kg^{-1} DM)
Annalena	161.7 ±1.96	641.5 ±7.76^{A}	0.27 ±0.005	1.08 ±0.018^{A}
Anuscha	248.0 ±4.87	811.3 ±15.9^{AB}	0.67 ±0.012	2.18 ±0.038^{AB}
Elfe	199.5 ±9.70	1832.6 ±89.1^{ABC}	0.67 ±0.012	6.18 ±0.108^{ABC}
Impala	496.1 ±14.0	2418.6 ±68.4^{ABC}	0.58 ±0.009	2.83 ±0.042^{ABC}
Marena	465.8 ±8.08	1848.3 ±32.1^{ABC}	0.31 ±0.006	1.23 ±0.022^{ABC}
Riviera	312.1 ±8.62	2853.0 ±78.83^{BC}	0.50 ±0.008	4.59 ±0.075^{BC}
Rosara	248.9 ±2.10	814.2 ±6.86^{C}	0.46 ±0.008	1.50 ±0.025^{C}

Note: Different letters indicate significant differences ($p < 0.001$)

Table 2 Cadmium and lead content in analyzed potato cultivars

Cultivar	Cd (mg.kg^{-1} FW)	Cd (mg.kg^{-1} DM)	Pb (mg.kg^{-1} FW)	Pb (mg.kg^{-1} DM)
Annalena	0.030 ±0.004	0.28 ±0.03A	0.143 ±0.016	1.31 ±0.14A
Anuscha	0.024 ±0.003	0.08 ±0.01AB	0.015 ±0.002	0.05 ±0.01AB
Elfe	0.064 ±0.008	0.25 ±0.03AB	0.370 ±0.043	1.47 ±0.17AB
Impala	0.006 ±0.001	0.02 ±0.002AB	0.428 ±0.049	1.40 ±0.16AB
Marena	0.028 ±0.004	0.26 ±0.03B	0.138 ±0.015	1.27 ±0.13AB
Riviera	0.021 ±0.002	0.10 ±0.01B	0.020 ±0.003	0.10 ±0.01B
Rosara	0.055 ±0.006	0.22 ±0.03B	0.325 ±0.042	1.29 ±0.17B

Note: Different letters indicate significant differences ($p < 0.001$)

Table 3 Relationships between monitored parameters

	TPC	AA	Cd	Pb
TPC	–			
AA	0.658***	–		
Cd	-0.426*	-0.391*	–	
Pb	-0.005	0.055	0.353	–

Note: * $p < 0.05$, *** $p < 0.001$

agrotechnical conditions, and genetic factors. The antioxidant activity also increases with the content of pigments, which is why it is higher in purple cultivars. Lee et al. (2016) indicate that the antioxidant activity is higher in purple cultivars than in white or yellow cultivars. According to Hamouz et al. (2011), higher antioxidant activity is associated with a higher content of anthocyanins.

Heavy metal content in potato tubers

Cadmium values in our samples ranged from 0.006 to 0.064 mg.kg^{-1} FW (0.02 to 0.278 mg.kg^{-1} DM) (Table 2).

According to Commission Regulation (EC) no. 1881/2006, the highest permissible amount of cadmium in fresh potatoes is 0.1 mg.kg^{-1}. This limit was not exceeded in monitored cultivars. According to World Health Organization (WHO), the recommended maximal concentration of Cd for potatoes is 0.05 mg.kg^{-1} fresh weight. This concentration was exceeded in cultivars Rosara and Elfe. Significant differences ($p < 0.001$) were between the Cd content of Annalena and Marena, Annalena and Riviera, and Annalena and Rosara. Dunbar et al. (2003) reported that potatoes contribute to 50% of dietary cadmium intake. Potato cultivars vary in their ability to accumulate Cd, as do many other plants. Ashrafzadeh et al. (2017) reported Cd content from 0.05 to 0.21 mg Cd.kg^{-1} DM depending on the cultivar. Jalali and Meyari (2016) reported about wide range of Cd values from 0.01 to 3.6 mg.kg^{-1} DM. Sanderson et al. (2019) reported that this parameter was from 0.01 to 0.22 mg Cd.kg^{-1} FW.

Lead values in our samples ranged from 0.015 to 0.370 mg.kg^{-1} FW (0.05 to 1.47 mg.kg^{-1} DM). According to Commission Regulation (EC) no. 1881/2006, the highest permissible amount of lead in fresh potatoes is 0.1 mg.kg^{-1}. This limit was exceeded in cultivars Annalena, Elfe, Impala, Marena, and Rosara. Significant differences ($p < 0.001$) were between the Pb content of Annalena and Riviera, and Annalena and Rosara. Musilová et al. (2016) determined the lead content in potato samples in the range from 0.020 to 0.630 mg.kg^{-1} FW. Musilová et al. (2017) reported from BDL (below detection limit) to 0.230 mg Pb.kg^{-1} FW. Mansour et al. (2009) reported about wide range of Pb values in potato tubers from 0.08 to 0.62 mg.kg^{-1} FW.

The relationships between the content of heavy metals and the content of polyphenols and antioxidant activity

In the natural environment, plants are exposed to various stress factors that are responsible for the overproduction of reactive oxygen species. One of these factors is the effect of heavy metals on plants (Zeneli et al., 2013). Environment and heavy metals significantly affect total phenolic values in potato tubers (André et al., 2009). The relationships between individual monitored parameters are presented in Table 3.

Positive correlations ($p < 0.001$) were determined between TPC and AA ($r = 0.658$). Negative correlations were determined ($p < 0.05$) between TPC and Cd content, and AA and Cd content. Musilová et al.

(2015) reported that a statistically significant positive correlation was confirmed between Cd content and total antioxidant capacity in potato cultivars. Musilová et al. (2009) reported a positive correlation between TPC and Cd content in potato tubers.

A weak correlation was determined between Cd and Pb content. Despite the fact that potatoes are less sensitive to a higher content of lead in the soil than to the content of cadmium, there is an increase in its content in the tubers, which is probably influenced by the synergistic effect of cadmium (Musilová et al., 2011).

Conclusions

Based on the results, it can be concluded that potato tubers are a source of bioactive compounds. Significant differences were determined in the TPC and AA of Annalena and Rosara, Anuscha and Rosara, Annalena and Riviera, and Anuscha and Riviera. Potatoes accumulate heavy metals, such as Cd and Pb, which are toxic to humans. The limit for Pb, set by Commission Regulation (EC) no. 1881/2006 was exceeded in cultivars Annalena, Elfe, Impala, Marena, and Rosara. Significant differences were determined in the Cd content of Annalena and Marena, Annalena and Riviera, and Annalena and Rosara. Significant differences were determined in the Pb content of Annalena and Riviera, and Annalena and Rosara. Positive correlations ($p < 0.001$) were determined between TPC and AA ($r = 0.658$). Negative correlations were determined ($p < 0.05$) between TPC and Cd content, and AA and Cd content. Results of this study will be useful for cultivar selection, and for future studies.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

Funding

This work was supported by grant Visegrad Fund for the research during which the presented knowledge was obtained.

Acknowledgements

This study was supported by the Operational Program Integrated Infrastructure within the project: Demand-driven Research for the Sustainable and Innovative Food, Drive4SIFood 313011V336, cofinanced by the European Regional Development Fund.

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

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Antibacterial properties of commercial lavender essential oil against some Gram-positive and Gram-negative bacteria

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Article Details:
Received: 2022-07-20
Accepted: 2022-08-16
Available online: 2022-11-30

DOI: https://doi.org/10.15414/ainhlq.2022.0023

Herbs and essential oils (EOs) have been used in medicine and veterinary, agriculture, the food industry, and cosmetology. Many EOs possess various biological properties, i.e. antibacterial, analgesic, anti-inflammatory properties, antioxidant, fungicide, larvicidal, antitumor activities, etc. Lavender oil is one of the most valuable aromatherapy oils. Its antibacterial and antifungal activities have been revealed in many studies. In the current study, the antibacterial properties of commercial lavender EO against some Gram-positive and Gram-negative bacteria were studied. To this intent, the antimicrobial susceptibility test was used (the Kirby–Bauer disk diffusion test for measuring zone diameters of bacterial growth inhibition). In the current study, Gram-negative strains such as *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 35218™), *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC® 27853™) and Gram-positive strains such as *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 29213™), methicillin-resistant (MRSA), mecA positive *Staphylococcus aureus* (NCTC® 12493), *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) (resistant to vancomycin; sensitive to teicoplanin) and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) were used. Results of this study revealed that resistant to the lavender EO were Gram-negative bacterial strains, such as *E. coli* (Migula) Castellani and Chalmers (ATCC® 25922™), *E. coli* (Migula) Castellani and Chalmers (ATCC® 35218™), *P. aeruginosa* (Schroeter) Migula (ATCC® 27853™) strains. The diameters of inhibition zones after the application of lavender EO were similar to control samples (96% ethanol). On the other hand, Gram-positive strains such as *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™), methicillin-resistant *S. aureus* (NCTC® 12493), *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) were sensitive to lavender EO. The highest diameters of inhibition zones after the application of lavender EO were observed for *E. faecalis* strains. This study demonstrates the potential of commercial lavender essential oil as an antibacterial agent and for use in the treatment of MRSA infection. The data contributes to the ongoing scientific investigation regarding the application of essential oils as natural antibacterial agents.

Keywords: commercial lavender essential oil, antibacterial activity, inhibition zones, Kirby-Bauer disc diffusion technique

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Introduction

Lavender essential oil (EO) has been used both cosmetically and therapeutically for centuries (Cavanagh and Wilkinson, 2002). It has been used as an anxiolytic drug, a mood stabilizer, a sedative, spasmolytic, antihypertensive, antimicrobial, and analgesic agent as well as a wound healing accelerator (Sasannejad et al., 2012). Several studies have investigated the antinociceptive, immunomodulatory and anti-inflammatory properties of compounds found in lavender EO (Silva et al., 2015). It is traditionally used in herbal medicine to relieve stress and anxiety confirmed by positive results in models of anxiety and depression using some animal and clinical studies (López et al., 2017). The two primary terpenoid constituents of lavender EO, linalool and linalyl acetate, may produce an anxiolytic effect in combination with inhibition of voltage-gated calcium channels, reduction of 5HT1A receptor activity, and increased parasympathetic tone (Malcolm and Tallian, 2018). Sasannejad et al. (2012) have studied the efficacy of lavender EO inhalation for the treatment of migraine in a placebo-controlled clinical trial. That study suggests that inhalation of lavender EO may be an effective and safe treatment modality in the acute management of migraine headaches (Sasannejad et al., 2012). Also, the current body of literature suggests apotential therapeutic benefit of lavender EO in wound healing. The studies of Samuelson et al. (2020) have demonstrated a faster rate of wound healing, increased expression of collagen, and enhanced activity of proteins involved in the tissue remodelling process in wounds treated with lavender EO.

The *Lavandula* genus includes about 40 different species and hundreds of varieties and hybrids. The three species most commonly grown are *L. angustifolia* Mill. (narrow-leaved lavender, usually medical), a species with the most significant industrial importance, *L. stoechas* (French lavender), *L. latifolia*, and their hybrids (Wińska et al., 2019). The composition of lavender EO is described in the review article written by Wińska et al. (2019). These researchers noted that the main components of lavender EO are R enantiomers of linalool (20–45%) and linalyl acetate (25 to 46%). The high content of these ingredients determines the quality of the oil. The content of other ingredients should be in the following ranges: limonene (>1.0%), eucalyptol (<2.5%), camphor (>1.2%), terpin-4-ol (0.1–6.0) %), lawandulol (<0.1%), lavandulyl acetate (<0.2%), and α-terpineol (>2.0%). Due to the incalculable influence on the scent, lavender oil should not contain too much ocymen, cineole, camphor, or terpin-4-ol (Wińska et al., 2019). The essential oil composition obtained from fresh flowers of thirteen new Ukrainian cultivars of *L. angustifolia* was analyzed by Pokajewicz et al. (2021). Eighty-two components were identified. Linalool and linalyl acetate were principal constituents of all of the samples and ranged from 11.4% to 46.7% and 7.4% to 44.2%, respectively (Pokajewicz et al., 2021).

The long-known antimicrobial actions of essential oils are now being extensively scientifically reviewed and applied in health and industry fields (Sienkiewicz et al., 2011, 2014). The antimicrobial activity of lavender EO against bacteria and fungi has long been established. Its anti-bacterial and anti-fungal activities can be explained by the presence of a main components such as linalool, linalyl acetate, lavandulol, geraniol, or eucalyptol (Białoń et al., 2019). Lavender EO also has antibacterial activity against clinical strains of bacteria isolated from patients with respiratory tract infections (Sienkiewicz et al., 2011). Moreover, the antibacterial activity of lavender EO may be accompanied by an immunostimulatory effect reducing the incidence of infections in patients with bacterial respiratory infections (Roller et al., 2009). Also, few studies have been carried out to elucidate the mechanism of its action to capitalize on its application in clinical settings (Yang et al., 2020).

In the current study, the antibacterial properties of commercial lavender EO provided by Polish essential oil manufacturers (Naturalne Aromaty sp. z o.o., Kłaj, Poland) against some Gram-positive and Gram-negative bacteria were studied. To this intent, the antimicrobial susceptibility test was used (the Kirby–Bauer disk diffusion test for measuring zone diameters of bacterial growth inhibition).

Material and methodology

Lavender essential oil

The lavender EO was provided by Polish essential oil manufacturers (Naturalne Aromaty sp. z o.o., Kłaj, Poland). The investigated sample did not contain additives or solvents and was confirmed to be natural by the manufacturers. The samples were stored in resalable vials at 5 °C in the dark but were allowed to adjust to room temperature prior to investigation. Geographical origins were excluded as information was mostly not available.

Determination of the antibacterial activity of plant extracts by the disk diffusion method

The testing of the antibacterial activity of lavender EO was carried out *in vitro* by the Kirby-Bauer disc

diffusion technique (Bauer et al., 1966). In the current study, Gram-negative strains such as *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 35218™), *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC® 27853™) and Gram-positive strains such as *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC® 29213™), methicillin-resistant (MRSA), mecA positive *Staphylococcus aureus* (NCTC® 12493), *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) (resistant to vancomycin; sensitive to teicoplanin) and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) were used.

The strains were inoculated onto Mueller-Hinton (MH) agar dishes. Sterile filter paper discs impregnated with lavender EO were applied over each of the culture dishes. Isolates of bacteria with lavender EO were then incubated at 37 °C for 24 h. The Petri dishes were then observed for the zone of inhibition produced by the antibacterial activity of lavender EO. A control disc impregnated with 96% ethanol was used in each experiment. At the end of the 24-h period, the inhibition zones formed were measured in millimetres using the vernier. For each strain, eight replicates were assayed (n = 8). The Petri dishes were observed and photographs were taken. The susceptibility of the test organisms to the lavender EO was indicated by a clear zone of inhibition around the discs containing the lavender EO and the diameter of the clear zone was taken as an indicator of susceptibility. Zone diameters were determined and averaged. The following zone diameter criteria were used to assign susceptibility or resistance of bacteria to the phytochemicals tested: Susceptible (S) ≥15 mm, Intermediate (I) = 10–15 mm, and Resistant (R) ≤10 mm (Okoth et al., 2013; Truchan et al., 2019).

Statistical analysis

Zone diameters were determined and averaged. Statistical analysis of the data obtained was performed by employing the mean ± standard error of the mean (S.E.M.). All variables were randomized according to the phytochemical activity of the lavender EO tested. All statistical calculation was performed on separate data from each strain. The data were analyzed using a one-way analysis of variance (ANOVA) using Statistica v. 13.3 software (TIBCO Software Inc., Krakow, Poland) (Zar, 1999).

Results and discussion

The antibacterial activity induced by lavender essential oil estimated as diameters of growth inhibition zones of examined Gram-positive and Gram-negative strains was presented in Figure 1 and 2.

Results of the current study revealed that Gram-negative strains, such as *E. coli* and *P. aeruginosa* were resistant to the lavender EO. The diameters of inhibition zones for *E. coli* (Migula) Castellani and Chalmers (ATCC® 25922™) strain after the application of lavender EO were similar (7.98 ±0.81 mm) compared to the 96% ethanol as control samples (8.85 ±0.91 mm). Similar results were obtained for *E. coli* (Migula) Castellani and Chalmers (ATCC® 35218™) strain. The diameters of inhibition zones after the application of lavender EO were (8.56 ±0.76 mm) compared to the 96% ethanol as control samples (8.98 ±0.88 mm). *P. aeruginosa* (Schroeter) Migula (ATCC® 27853™) strain was also resistant to the lavender EO. The diameters of inhibition zones after the application of lavender EO were (7.12 ±0.81 mm) compared to the 96% ethanol as control samples (7.78 ±0.91 mm) (Figure 1).

Gram-positive strains were sensitive to the lavender EO compared to the Gram-negative strains. *S. aureus* strains exhibited *intermedia*te activity to the lavender EO. *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™) strain was less sensitive then *S. aureus* (NCTC® 12493). Diameters of inhibition zones after application of lavender EO were (13.44 ±0.56 mm) compared to the 96% ethanol as control samples (8.56 ±0.75 mm) for *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™) strain and (16.10 ±0.69 mm) compared to the 96% ethanol as control samples (9.12 ±0.95 mm) for *S. aureus* (NCTC® 12493) strain. The increase of diameters of inhibition zones after the application of lavender EO was 57% ($p < 0.05$) and 76.5% ($p < 0.05$) for *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™) and *S. aureus* (NCTC® 12493) strains, respectively compared to the control samples (96% ethanol) (Figure 1).

E. faecalis strains were more sensitive to lavender EO (Figure 1). Diameters of inhibition zones after application of lavender EO were (21.78 ±0.71 mm) compared to the 96% ethanol as control samples (9.15 ±0.99 mm) for *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) strain and (23.15 ±0.98 mm) compared to the 96% ethanol as control samples (8.92 ±0.91 mm) for *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) strain. The increase of diameters of inhibition zones after the application of lavender EO was 138% ($p < 0.05$) and 159.5% ($p < 0.05$) for

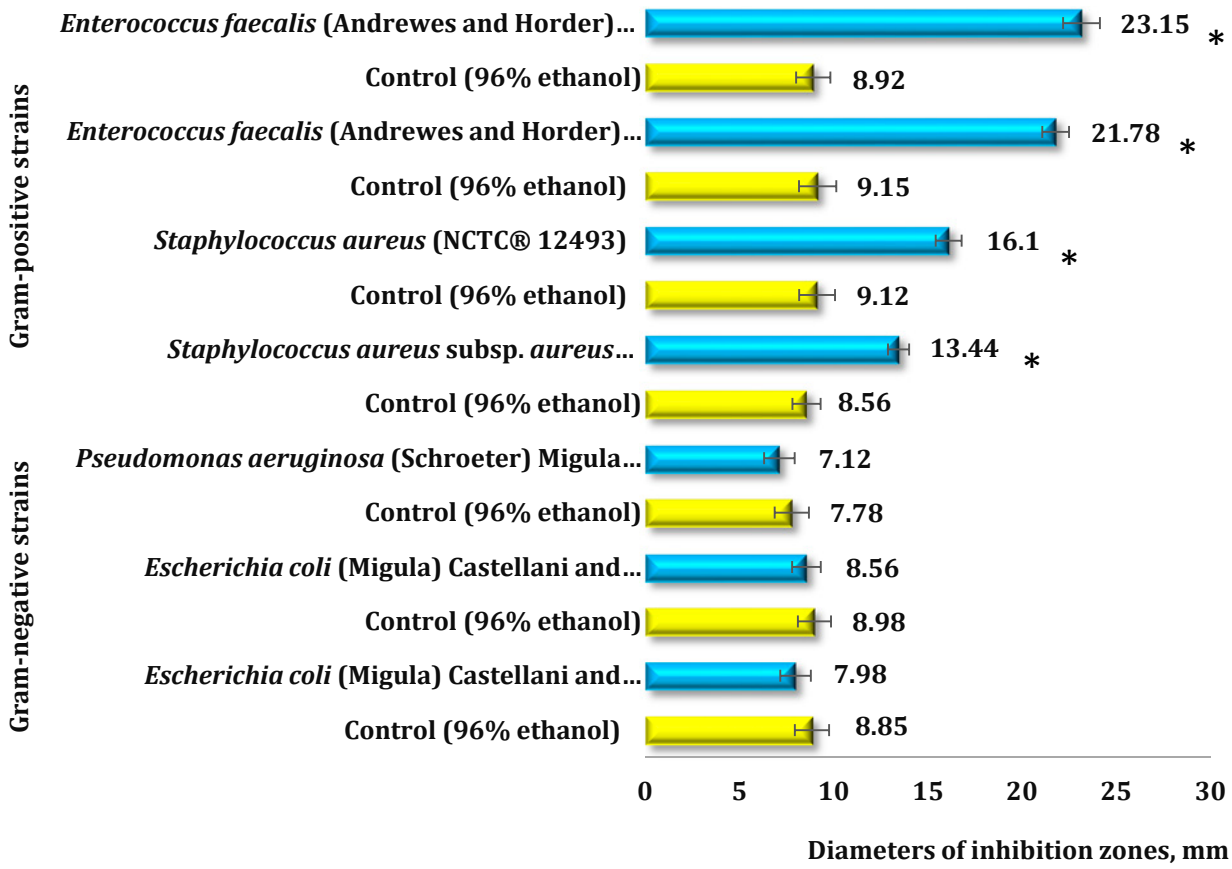

Figure 1 The antibacterial activity induced by lavender essential oil estimated as diameters of growth inhibition zones of examined Gram-positive and Gram-negative strains. The data were presented as the mean ± the standard error of the mean (S.E.M.)
* denote significant differences between the control (96% ethanol) and lavender EO ($p < 0.05$)

E. faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) strains, respectively (Figure 1).

Detailed photos regarding the zones of inhibition by the lavender EO against Gram-positive and Gram-negative bacterial strains were recorded and presented in Figure 2.

In line with our previous studies according to the antibacterial potential of different plant extracts and EOs, in the current study, we examined the antibacterial potential of commercial lavender EO against Gram-positive and Gram-negative bacterial strains. Resistant to the lavender EO were Gram-negative bacterial strains, such as *E. coli* (Migula) Castellani and Chalmers (ATCC® 25922™), *E. coli* (Migula) Castellani and Chalmers (ATCC® 35218™), *P. aeruginosa* (Schroeter) Migula (ATCC® 27853™) strains. The diameters of inhibition zones after the application of lavender EO were similar to control samples (96% ethanol). On the other hand, Gram-positive strains such as *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™), methicillin-resistant *S. aureus* (NCTC® 12493), *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) were sensitive to lavender EO. The highest diameters of inhibition zones after the application of lavender EO were observed for *E. faecalis* strains (Figure 1 and 2).

Lavender EO has antibacterial activity against bacterial strains, as reported in many studies. For example, Roller et al. (2009) have compared the antimicrobial efficacy of several lavender oils from *Lavandula angustifolia*, *L. latifolia*, *L. stoechas*, and a necrodane-rich *L. luisieri*, used singly and in combination, on methicillin-sensitive and methicillin-resistant *S. aureus* (MSSA and MRSA). All four lavender oils inhibited the growth of both MSSA and MRSA by direct contact but not in the vapour phase. Inhibition zones ranged from 8 to 30 mm in diameter at oil doses ranging from 1 to 20 µL, respectively, demonstrating a dose-response. At any single dose, the extent of inhibition was very similar irrespective of the chemical composition

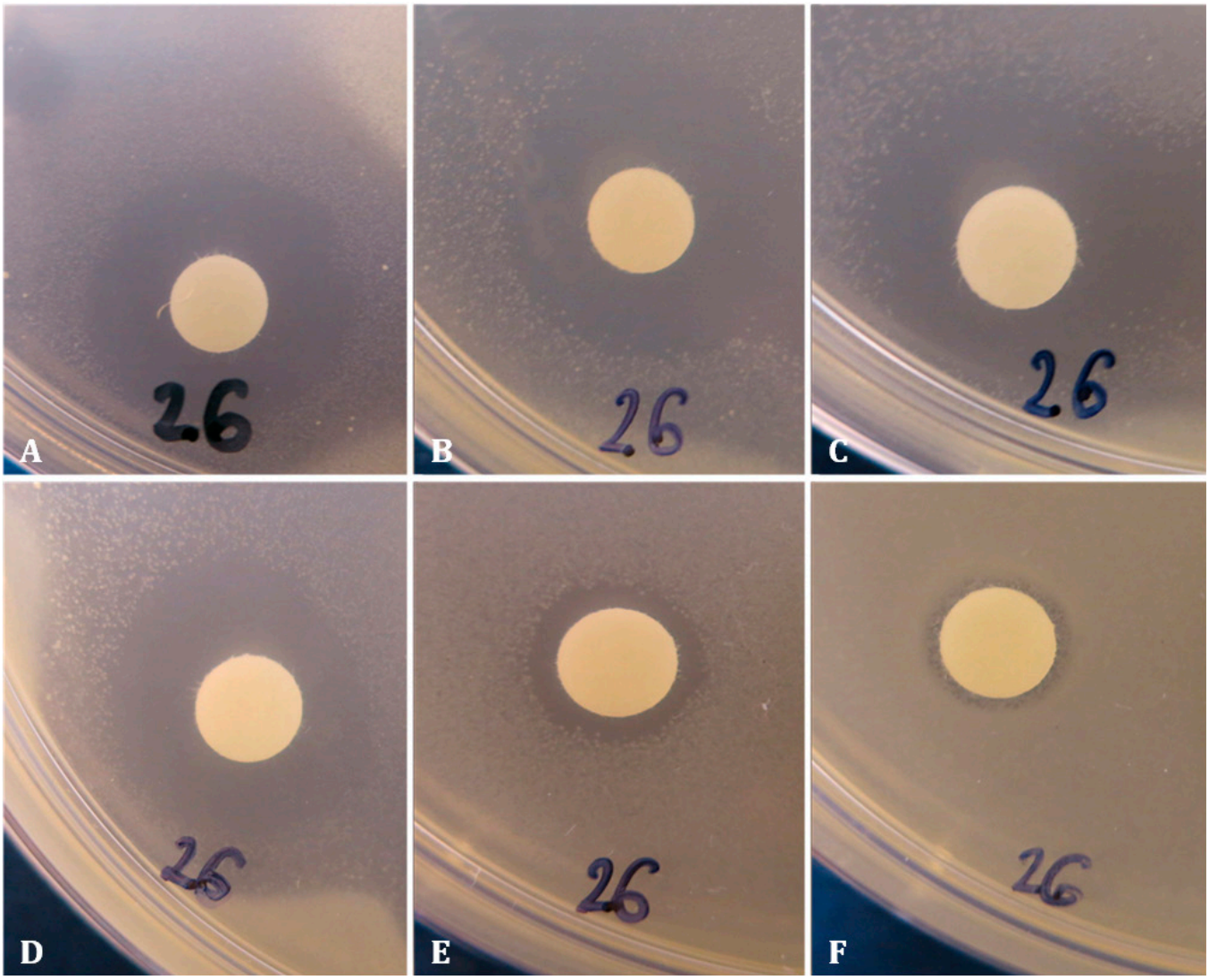

Figure 2 Inhibition growth zones induced by lavender essential oil against *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) (A), *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) (B), *Staphylococcus aureus* subsp. aureus Rosenbach (ATCC® 29213™) (C), *Staphylococcus aureus* (NCTC® 12493) (D), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 25922™) (E), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC® 35218™) (F)

of the oils or the strain of *S. aureus* used. Several binary combinations of the oils were tested, and the results showed that the necrodane-rich *L. luisieri* oil interacted synergistically with *L. stoechas* (high in 1,8-cineole, fenchone, and camphor) and *L. angustifolia* (rich in linalool and linalyl acetate) to produce larger inhibition zones than those produced using each oil individually (Roller et al., 2009).

Lavender EO could be a promising candidate for an efficient enhancer of conventional antiseptics. Kwiatkowski et al. (2019) have investigated the impact of lavender EO on octenidine dihydrochloride (OCT) efficiency towards methicillin-resistant *S. aureus* strains (MRSA). The lavender EO increased the OCT's susceptibility against MRSA strains. Subsequent FTIR analysis revealed cellular wall modifications in MRSA strain cultured in media supplemented with OCT or lavender EO/OCT (Kwiatkowski et al., 2019).

The chemical composition of lavender (*Lavanda angustifolia* L.) EO and some by-products derived from its production (residual water, residual herbs), as well as their *in vitro* antimicrobial activity, were assessed by Ciocarlan et al. (2021). The main constituents of EOs are monoterpenes (84.08–92.55%), followed by sesquiterpenes (3.30–13.45%), and some aliphatic compounds (1.42–3.90%). The high-performance liquid chromatography analysis allowed the quantification of known triterpenes, ursolic, and oleanolic acids, in freshly dried lavender plants and the residual by-products after hydrodistillation

of the essential oil. The lavender essential oil showed good antibacterial activity against *Bacillus subtilis*, *Pseudomonas fluorescens*, *Xanthomonas campestris*, *Erwinia carotovora* at 300 μg.mL^{-1} concentration, and *Erwinia amylovora*, *Candida utilis* at 150 μg.mL^{-1} concentration, respectively. Lavender plant material but also the residual water and ethanolic extracts from the solid waste residue showed high antimicrobial activity against *Aspergillus niger*, *Alternaria alternata*, *Penicillium chrysogenum*, *Bacillus* sp., and *Pseudomonas aeroginosa* strains, at 0.75–6.0 μg.mL^{-1}, 0.08–0.125 μg.mL^{-1}, and 0.05–4.0 μg.mL^{-1}, respectively (Ciocarlan et al., 2021).

Lavender EOs could find potential applications in food biopreservation and surface decontamination, even in hospitals. Tardugno et al. (2019) evaluated the antimicrobial activity of EOs of four cultivars (cv) of *Lavandula* × *intermedia* (Abrialis, Alba, Rinaldi Ceroni (R.C.) and Sumiens) against *Listeria monocytogenes* (24 strains) and *Salmonella enterica* (10 food strains). Minimal inhibitory concentrations (MIC) ≥10.0 μL.mL^{-1} inhibited *Salmonella* (cv. R.C. was the most active); MIC of 0.3 μL.mL^{-1} for cv. Abrialis and cv. R.C. inhibited *L. monocytogenes*, revealing noticeable activity, especially on clinical strains. Particularly cv. Abrialis and cv. R.C. showing the highest antimicrobial activity, were rich in the specific constituents: linalool (38.17 and 61.98%), camphor (8.97 and 10.30%), 1,8-cineole (6.89 and 8.11%, respectively) (Tardugno et al., 2019).

The anti-microbial effects of two different lavender oils such as commercial lavender oil and essential lavender oil from the Crimean Peninsula on a mixed microbiota from facial skin were assessed by Białoń et al. (2019). The composition and properties of the studied oils were significantly different. The commercial lavender oil (Etja, Poland) contained 10% more linalool and linalyl acetate than the Crimean lavender oil. Both oils also had different effects on the mixed facial skin microbiota. The Gram-positive bacilli were more sensitive to commercial lavender EO, and Gram-negative bacilli were more sensitive to Crimean lavender EO. However, neither of the tested oils inhibited the growth of Gram-positive cocci. The tested lavender oils decreased the cell number of the mixed microbiota from facial skin, but commercial lavender EO showed higher efficiency, probably because it contains higher concentrations of monoterpenoids and monoterpenes than Crimean lavender oil does (Białoń et al., 2019).

The laboratory and clinical efficacy of lavender oil in the treatment of recurrent aphthous ulceration were revealed by Altaei (2012). Animals treated with lavender oil showed a significant ulcer size reduction, increased rate of mucosal repair, and healing within 3 days of treatment compared to baseline and placebo groups (2–3 days (90%), 4 days (10%)). Lavender oil showed a broad antibacterial activity against all tested strains; it exhibited significant inhibition on tested bacteria where the value of zone of inhibition ranged from 14.5–24.0 mm vs. Streptomycin (25 μg.disc^{-1}) 12–22 ±0.5 mm; MIC was >6.4–36 mg.ml^{-1}. Patients with recurrent aphthous ulceration treated with lavender oil showed a significant reduction in inflammation level, ulcer size, and healing time, from 2–4 days (2 days (40%), 3 days (50%), 4 days (10%)), and pain relief mostly from the first dose, compared to baseline and placebo (Altaei, 2012).

The combined use of two naturally derived compounds, sodium alginate and lavender essential oil, for the production of bioactive nanofibrous dressings by electrospinning, and their efficacy for the treatment of skin burns induced by midrange ultraviolet radiation (UVB) was demonstrated by Hajiali et al. (2016). These researchers have demonstrated that the engineered dressings reduce the risk of microbial infection of the burn since they stop the growth of *Staphylococcus aureus*. Furthermore, they can control and reduce the inflammatory response that is induced in human foreskin fibroblasts by lipopolysaccharides, and in rodents by UVB exposure (Hajiali et al., 2016).

The products derived from the *Lavandula pubescens* Decne (LP), including the EO, have been used in Traditional Arabic Palestinian Herbal Medicine for centuries as therapeutic agents. The EO is traditionally believed to have sedative, anti-inflammatory, antiseptic, anti-depressive, anti-amnesia, and anti-obesity properties. Ali-Shtayeh et al. (2020) have assessed the *in vitro* bioactivities associated with the aerial parts of LP plants and analyzed them for their antioxidant, antimicrobial, anticholinesterase, and anti-lipase activities. The EO also demonstrated high antibacterial activity with the highest susceptibility observed for *S. aureus* with 95.7% inhibition. The EO was shown to exhibit strong inhibitory activity against *Candida albicans* (MIC 0.47 μL.mL^{-1}). The EO was also shown to possess strong anti-dermatophyte activity against *Microsporum canis*, *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Epidermophyton floccosum* (EC_{50} 0.05–0.06 μL.mL^{-1}). The high antioxidant, enzyme inhibitory, and antimicrobial potentials of the EO can, therefore, be correlated with its high content of monoterpenes, especially carvacrol, as shown by its comparable bioactivities indicators results (Ali-Shtayeh et al., 2020).

The chemical composition of *Lavandula angustifolia* Mill. EO collected during four consecutive years of growth were the aims of the work of Najar et al. (2022). The antibacterial activities of the EOs were assessed on three Gram-positive bacteria strains: *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* VAN B V 583 E, and *Listeria monocytogenes*, together with three Gram-negative bacteria strains: *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 15325, and *Salmonella enterica* ser. Typhimurium ATCC 14028. Gram-negative bacteria were found to be less inhibited by oils derived from plants each year with maximum MIC values of 1:32 for *Escherichia coli* for fourth-year plants. The most sensitive bacterium was *Listeria monocytogenes* (a maximum MIC of 1:128 and MBC of 1:64 for the EOs of fourth-year plants), while the most resistant was *Pseudomonas aeruginosa* (a maximum MIC of 1:16 and MBC of 1:8 for the EO of fourth-year plants). Considering the age of the plants from which the oil was extracted, the ones that showed a higher inhibitory and bactericidal activity were those of the fourth year, followed by (in decreasing order) those of the third year, the second year, and finally the first year. Comparing these data with the GC–MS analyses, the trend of increased inhibitory efficacy against bacteria demonstrated as the plants aged is superimposed by the trend of an increased relative percentage of linalool (19.3, 23.1, 27.6, and 34.2 for each year), which was highly correlated with the *Escherichia coli* ATCC 15325 activity (correlation coefficient: 0.9). Linalool has shown a significant effect on *P. fluorescens* with 1.25 and 2.5 $\mu L.mL^{-1}$ of the MIC and MBC, respectively. The EO extracted from the oldest plants evidenced higher activity on the studied strains, with more sensitivity on the Gram-positive ones. Tuscan lavender EO, especially that obtained from four-year-old plants, is of great interest for its potential industrial applications and constitutes an example of the valorization of marginal Tuscan land and good-quality production (Najar et al., 2022).

L. angustifolia EO can stimulate the human innate macrophage response to bacteria that are responsible for one of the most important nosocomial infections and might suggest the potential development of this plant extract as an anti-inflammatory and immune regulatory coadjutant drug. Giovannini et al. (2016) have investigated, by transcriptional analysis, how an *L. angustifolia* EO treatment influenced the macrophage response to *Staphylococcus aureus* infection. The results of these researchers showed that the treatment increased the phagocytic rate and stimulates the containment of intracellular bacterial replication by macrophages. This stimulation is coupled with the expression of genes involved in reactive oxygen species production. Moreover, the EO treatment balanced the inflammatory signaling induced by *S. aureus* by repressing the principal pro-inflammatory cytokines and their receptors and inducing the heme oxygenase-1 gene transcription (Giovannini et al., 2016). The lavender EO extracted at the beginning of the flowering period is a potent inhibitor of the synthesis of four pro-inflammatory cytokines IL-6, IL-8, IL-β, and TNFα of THP-1 macrophages (Pandur et al., 2021). This supports the relevance of the collection of lavender flowers from the early blooming period for essential oil production and for utilization as an anti-inflammatory treatment (Pandur et al., 2021).

Conclusions

In summary, this study provides insight into the *in vitro* antibacterial activity of commercial lavender EO against Gram-negative strains such as *E. coli* (Migula) Castellani and Chalmers (ATCC® 25922™), *E. coli* (Migula) Castellani and Chalmers (ATCC® 35218™), *P. aeruginosa* (Schroeter) Migula (ATCC® 27853™) and Gram-positive strains such as *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™), methicillin-resistant (MRSA) *S. aureus* (NCTC® 12493), *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) (resistant to vancomycin; sensitive to teicoplanin) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™). Results of the current study revealed that resistant to the lavender EO were Gram-negative bacterial strains, such as *E. coli* (Migula) Castellani and Chalmers (ATCC® 25922™), *E. coli* (Migula) Castellani and Chalmers (ATCC® 35218™), *P. aeruginosa* (Schroeter) Migula (ATCC® 27853™) strains. The diameters of inhibition zones after the application of lavender EO were similar to control samples (96% ethanol). On the other hand, Gram-positive strains such as *S. aureus* subsp. *aureus* Rosenbach (ATCC® 29213™), methicillin-resistant *S. aureus* (NCTC® 12493), *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 51299™) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC® 29212™) were sensitive to lavender EO. The highest diameters of inhibition zones after the application of lavender EO were observed for *E. faecalis* strains. This study demonstrates the potential of commercial lavender essential oil as an antibacterial agent and for use in the treatment of MRSA infection. The data contributes to the ongoing scientific investigation regarding the application of essential oils as natural antibacterial agents.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

Funding

The authors would like to extend their sincere appreciation to The International Visegrad Fund for supporting our study.

Acknowledgements

This work was supported by Pomeranian University in Słupsk (Poland) in cooperation with M.M. Gryshko National Botanic Garden, National Academy of Science of Ukraine (Kyiv, Ukraine).

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

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Phenological growth stages according to the BBCH scale *Elaeagnus multiflora* Thunb.

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Article Details:
Received: 2022-07-24
Accepted: 2022-08-21
Available online: 2022-11-30

DOI: https://doi.org/10.15414/ainhlq.2022.0024

Phenology is a key trait of plants of all species, as it determines their season and duration of growth and reproduction, as well as their ability to capture variable resources. Understanding the phenology of *Elaeagnus multiflora* Thunb. a rare but promising fruit and medicinal plant of Ukraine, namely the codification of the stages of seasonal development, according to the international BBCH scale, is important for the evaluation of breeding material and the development of new varieties, improving the technological qualities of fruits. In the climatic conditions of Ukraine (M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine, Kyiv), the studied genotypes of *E. multiflora* go through a full cycle of development. Plants begin the growing season with the development of leaves and shoots. According to the international BBCH scale, they clearly distinguish eight of the ten main stages of seasonal development, in particular: the development of buds (Principal growth stage 0), leaves (Principal growth stage 1), shoots (Principal growth stage 3), inflorescence emergence (Principal growth stage 5), flowering (Principal growth stage 6), fruit development (Principal growth stage 7), fruit ripening (Principal growth stage 8) and senescence and the onset of dormancy (Principal growth stage 9). The proposed BBCH scale for characterizing the phenological stages of *E. multiflora* plants can be used to guide the growers as to when to carry out orchard management practices such as canopy training and pruning, nutrient and water application, pest and disease control and post-harvest processing. Correct identification of phenological stages is of great importance for the characterization and management of *E. multiflora*. Thus, this study will ensure the dissemination of knowledge about *E. multiflora* cultivars among growers and researchers.

Keywords: *Elaeagnus multiflora*, BBCH scale, developmental stages, phenology, phenophase

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Introduction

Observations of the seasonal development of plants, both wild and introduced, have been carried out since ancient times using various techniques (Meier et al., 2009). In the middle of the last century, the need for a single, international standard for displaying the phenological stages of plant growth, regardless of taxonomic affiliation and research region, became apparent (Cautín, Agustí, 2005; Zhao et al., 2019). Based on the numerical code of Zadoks et al. (1974), the BBCH scale was developed, and then the expanded BBCH scale (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) (Berning et al., 1987a, b, c; Bleiholder et al., 1986, 1989; Lancashire et al., 1991; Hack et al., 1992). Since then, the BBCH scale has been widely used to record and describe the phases of the seasonal development of plants of various types in different climatic zones, in particular, fruit plants, namely: *Cydonia oblonga* Mill. (Martínez-Valero et al., 2001), *Olea europea* L. (Sanz-Cortés et al., 2002), *Prunus armeniaca* L. (Pérez-Pastor et al., 2004), *Actinidia deliciosa* (A.Chev.) C.F.Liang & A.R.Ferguson (Salinero et al., 2009), *Diospyros kaki* Thunb. (García-Carbonell et al., 2002; Guan et al., 2021), *Diospyros virginiana* L. (Grygorieva et al., 2010), *Mangifera indica* L. (Hernández Delgado et al., 2011), *Mespilus germanica* L. (Atay, 2013), *Persea americana* Mill. (Alcaraz et al., 2013), *Ziziphus mauritiana* Lamk. (Krishna et al., 2019), *Prunus avium* L. (Fadón et al., 2018), *Pseudocydonia sinensis* C.K. Schneid. (Grygorieva et al., 2018a), *Malus domestica* Borkh (Martínez-Valero et al., 2019), *Prunus dulcis* (Mill.) D.A.Webb (Sakar et al., 2019), *Myrtaceae* species (Guollo et al., 2020), *Cornus* L. (Klymenko and Ilyinska, 2021), *Cornus sessilis* Torr. ex Durand (Klymenko et al., 2021). In fruit growing, detailed codification of seasonal stages of plant growth is important for evaluating breeding material and breeding new varieties, improving the technological qualities of fruits; it is a good reference for growers and scientists who need uniform selection criteria, as well as the development of management methods for the expansion of commercial cultivation.

The genus *Elaeagnus* L. (Elaeagnáceae Juss., nom. cons.) includes almost 90 species distributed in Asia, southern Europe, North America, and South-Eastern Australia (Qin and Gilbert, 2007). The greatest species diversity, including 55 endemic species, is concentrated in China (Qin and Gilbert, 2007; Sun and Lin, 2010). Plants of many species (for example *E. angustifolia* L., *E. commutata* Bernh., *E. pungens* Thunb., *E. umbellata* Thunb.) have economic value and are used as a fruit, medicinal (in traditional medicine), honey-bearing or decorative (Qin and Gilbert, 2007; Lachowicz et al., 2020; Nazir et al., 2020; Bieniek et al., 2022; Yang et al., 2022) plants. *Elaeagnus multiflora* Thunb. (cherry elaeagnus, cherry silverberry, gumi) has Japanese origin and also it belongs to the number of promising fruit plants with high nutritional and medicinal potential.

The natural range of this species includes China, Japan, Korea, and the Kuril Islands; introduced into the culture in North (USA) and South America (Colombia, Brazil), as well as in 11 European countries (*Elaeagnus*, 2021). In its homeland, *Elaeagnus multiflora* has been cultivated as an ornamental, food, and medicinal plant for several centuries (Sakamura and Suga, 1987; You et al., 1994; Lee et al., 2007). Plants form thickets and sparse forests in the lowlands and the mountains from sea level to 1,800 m above sea level. r. m. They can grow on poor soils, due to symbiosis with nitrogen-fixing microorganisms living in root nodules (Qin and Gilbert, 2007; Sun and Lin, 2010), resistant to drought and frost. They bear fruit regularly and abundantly. Fruits contain carbohydrates, organic acids, amino acids, vitamins C and F, biominerals, polyphenols, flavonoids, carotenoids, chlorophylls and tocopherols, macro- and microelements, which ensures their high nutritional value (Vasyuk and Moroz, 2006; Bieniek et al., 2017, 2022). Biologically active compounds are also present in the bark, leaves, flowers, and seeds (Shin et al., 2008; Patel, 2015; Lachowicz et al., 2020).

In Ukraine, cherry elaeagnus is grown in some botanical gardens and as a fruit plant on private plots, in particular in the Lviv region (Vasyuk and Moroz, 2005; *Elaeagnus*, 2021). In M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine, (NBG) cherry elaeagnus was introduced in 1980–1982. The modern collection of *E. multiflora* in the NBG includes 45 genotypes. In the climatic conditions of Kyiv, the variability of the morphometric parameters of fruits was investigated (Grygorieva et al., 2018b), their biochemical composition, and also for the first time, oil was isolated from fruits, its quantitative and qualitative composition was determined, and it was established that the seeds and pulp of fruits contain irreplaceable of acids (linoleic and linolenic) is significantly higher than in the fruits of sea buckthorn (*Hippophaë* L. sp.) (Vasyuk and Moroz, 2006). Attention is also paid to the peculiarities of vegetative growth and generative development, winter and drought resistance, the nature of fruiting, seed, and vegetative propagation of plants. A phenological study of *E. multiflora* was conducted based on the use of fairly general and not very detailed methods of Beidemann and Lapin and Sidneva, which

are widespread mostly in Eastern Europe (Vasyuk and Moroz, 2005).

These data are important, but they lack detailed information, as only the main stages of seasonal rubber development are described. Therefore, a detailed phenological characterization is needed, which covers the entire life cycle of *E. multiflora* and which meets modern international standards. Thus, the purpose of our work is to describe and codify the seasonal development of *E. multiflora*, according to the international BBCH scale.

Material and methodology

Research area

The experiment was conducted in NBG, Kyiv (latitude: 50° 27.28‘ N; longitude: 30° 31.428‘ E, altitude: 197 m above sea level), which is located in the north of the central part of Ukraine in the middle course of the Dnipro; the climate is continental with mild winters and warm summers – Dfb, according to the Köppen-Geiger classification (Peel et al., 2007).

Biological material

30-year-old genotypes of *Elaeagnus multiflora* growing in the M.M. Gryshko National Botanical Garden. Ten healthy trees were randomly selected.

Phenological studies

Phenological behavior was assessed by observation dates and photographs. Seasonal phases of development are classified based on the BBCH (Biologische Bundesantalt, Bundessortenamt und Chemische Industrie) scale (Hack et al., 1992; Meier et al., 2009). A two-digit numbering system was used, where the first digit corresponded to the primary stage of growth, and the second to the secondary one. Data were recorded at intervals of three days. Throughout the experiment (April–November 2021), basic meteorological data were monitored.

Results and discussion

Phenology is a key trait of plants of all species, as it determines their season and duration of growth and reproduction, as well as their ability to capture variable resources (Schwartz, 2003; Chuine, 2010).

Elaeagnus multiflora is a deciduous shrub or small tree with a different crown shape (from upright to spreading), rusty-red young branches, abundantly covered with peltate trichomes (scales) and with

Figure 1 *Elaeagnus multiflora* Thunb. deciduous shrub

stellate hairs along the midvein of young leaves (Figure 1).

The biological minimum of *E. multiflora*, like many other fruit plants of the temperate zone, is ≥5.0 °C (Chmielewski and Köhn, 2000; Vasyuk and Moroz, 2005). In the climatic conditions of Ukraine (NBG), the genotypes of *E. multiflora* go through a full cycle of development. Plants usually start vegetation in the first decade of April with the development of leaves and shoots when the sum of effective temperatures is 36–59 °C (Table 1, Figure 1).

Principal growth stage 0. Bud development

E. multiflora is characterized by two types of buds such as vegetative (developing on proleptic, epicormic, and sylleptic shoots) and complex, vegetative-generative, which are formed on replacement proleptic shoots.

The development of vegetative buds of gum occurs in the same way as in other woody plants of the temperate zone. At rest (phenophase 00), the buds are round, half-open, and covered with brown scales. At the beginning of swelling (phenophase 01), buds increase in size. At the end of swelling (phenophase 03), they reach their final size, after which brown bud scales

Table 1 Phenological growth stages of *Elaeagnus multiflora* Thunb. according to the BBCH scale

BBCH Code	Description
	Principal growth stage 0: Bud development
00	bud dormancy
01	beginning of bud swelling
03	end of bud swelling
07	budding begins: the first silvery-green tips of the leaves are just visible
09	silvery-green leaf tips about 3 mm above bud scale
	Principal growth stage 1: Leaf development
10	first leaves separating: silvery-green leaf tips about 10 mm above the bud scales
11	the first young leaves unfolded, the rest of the leaves have not completely unfolded yet
15	more leaves unfolded, but not yet at full size; petioles visible
19	the first leaves have reached the characteristic size for the species
	Principal growth stage 3: Shoot development
31	beginning of shoot growth: axes of developing shoots visible
32	20% of the expected typical shoot length (annual shoot) achieved
34	50% of the expected typical shoot length (annual shoot) achieved
39	90% of the expected typical shoot length (annual shoot) achieved
	Principal growth stage 5: Inflorescence emergence
51	reproductive buds swelling in leaf axils: buds closed, greenish-brown scales visible
54	bud burst: scales separated, the silver-white tops of the buds are visible
55	sepals visible, but still united, flower pedicel elongating (green bud)
56	flowers still closed; sepals slightly begin to separate
59	first flowers form a hollow ball
	Principal growth stage 6: Flowering
60	first flowers open (sporadically)
61	beginning of flowering: about 10% of flowers open
65	full flowering: 50% of flowers open, the calyxes of the first flowers dry
67	flowering finishing; the calyxes of many flowers have dried up
69	the end of flowering: the calyxes of all flowers have dried up; fruit set is visible
	Principal growth stage 7: Fruit development
71	fruit set, beginning of ovary growth
72	fruit at 20% of final size
75	fruit at 50% of final size
79	fruit at 90% of final size, green
	Principal growth stage 8: Fruit and seed maturation
81	the beginning of fruit ripening: the color of the fruit changes from green to yellow
85	the color of the fruits progresses: it acquires a red color characteristic of the species
87	increasing color intensity; 80% of the fruits have reached technical ripeness; the flesh is crisp and sweet with typical taste and correct firmness
89	fruits colour fully developed. fruit ripe for consumption, the flesh is crisp and sweet with typical taste and correct firmness

Continuation of Table 1

BBCH Code	Description
	Principal growth stage 9: Senescence and beginning of the rest period
91	shoot growth is complete, the terminal bud is developed, but the leaves are still green
92	change in leaf color, green color started to disappear
93	the beginning of falling leaves
95	leaves at 50% fallen
97	dropping of all leaves
99	the beginning of winter dormancy

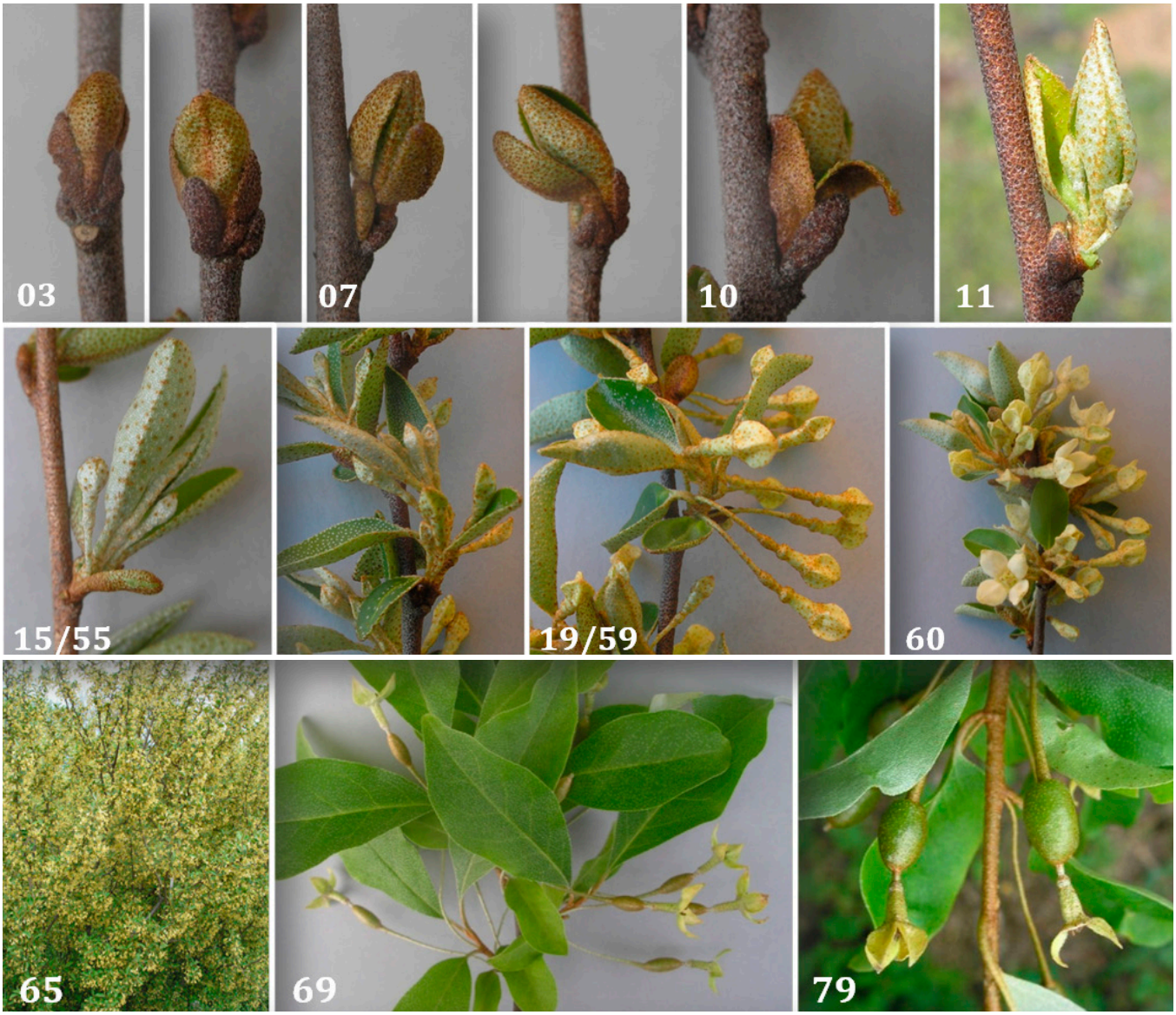

Figure 2a Phenological stages of the *Elaeagnus multiflora* Thunb. plants

Figure 2b Phenological stages of the *Elaeagnus multiflora* Thunb. plants

separately. Next, the silver-green tips of the leaves become noticeable (phenophase 07), as well as the green adaxial surface of the bud scales, thanks to which they can perform the function of photosynthesis, which intensifies the further development of the leaves. At the end of phenophase 09, all bud scales are opened, and the tips of the leaves reach about 3 mm in length.

Principal growth stage 1: Leaf development

The formation of leaves of vegetative shoots lasts from mid-April to August, and the development and growth of leaves of replacement shoots continue for about two months from mid-April to the end of May.

The first to begin development (phenophases 10, 11) are the three prophyllum. They differ from real leaves in a much smaller size, as in many other types of fruit plants. The full development of the first typical leaves (phenophase 19) completes "Principal growth stage 1".

Typical leaves of *E. multiflora* are elliptic or ovate to obovate-oblong, 3–10 × (1–)1.2–5 cm, abaxially with densely overlapping white and scattered pale brown scales, scales shallowly umbonate, adaxially stellate-pilose while young, lateral veins 5–7 per side of the midrib, base obtuse to cuneate, apex obtuse to acute or bluntly acuminate. Petiole 4–6 mm, brown scaly (Figure 3).

Principal Growth Stage 3: Shoot development (sprout from the terminal bud)

The study of shoot growth dynamics is an important indicator, as it allows us to assess the compliance of new environmental conditions with the needs of introduced plants and the course of adaptation processes.

Elaeagnus multiflora is characterized by proleptic vegetative and vegetative-generative shoots that develop from overwintering buds. In addition, epicormic vegetative shoots formed from dormant buds are also characteristic, and on which sylleptic enrichment shoots develop from buds with no period

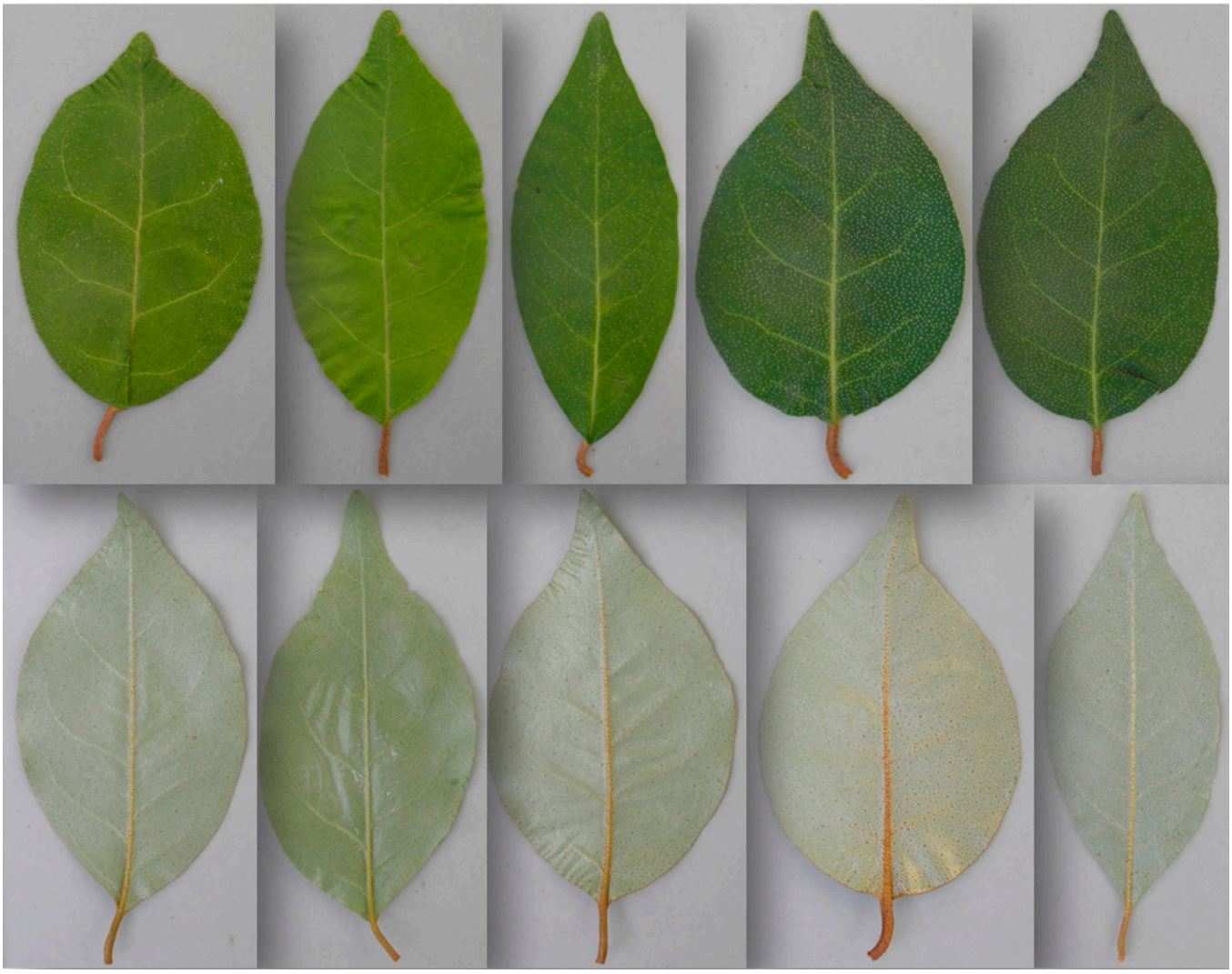

Figure 3 Leaves of *Elaeagnus multiflora* Thunb.

of growth dormancy, as in other fruit plants (Negrón et al., 2014).

Stems of one-year shoots are light brown or reddish-brown, covered with scales (Figure 1), perennial, gray-brown, very branched, and sometimes have thorns. Replacement shoots are the first start growing usually in the second half of April; they are short, 14.0–18.0 cm long. The development of vegetative shoots becomes noticeable 10–12 days later and lasts until the end of August. At the end of the season (phenophase 39), they are thickened, strengthened, gray-green, densely covered with white spots, 128.2–160.3 cm long. Sylleptic shoots of enrichment are formed in mid-July on epicormic vegetative shoots (in the middle part of their length) and are characterized by slow growth. They are short (about 4.0–6.0 cm long), and their tip is often transformed into a thorn. We studied the development of vegetative shoots.

Principal Growth Stage 5: Inflorescence emergence

Many species of the *Elaeagnus* genus are characterized by racemes or umbel-like inflorescences (Sun and Lin, 2010). In *E. multiflora*, flower buds develop one or two at a time (occasionally) in the axils of the lower leaves of the replacement shoots. They are covered with peltate trichomes, like the whole plant. On one shoot, flower buds are formed gradually, synchronously with its growth and development. Therefore, flowers and buds of various degrees of development are observed at the same time. The period of development of flower buds lasts from the end of April to the middle of May, approximately 2–3 weeks.

Principal growth stage 6: Flowering

The flowers of *E. multiflora* are regular, bisexual, fragrant, 4-membered, without petals, as in other

Figure 4 Variation in the shape and color of perianths of *Elaeagnus multiflora* Thunb.

species of the Elaeagnaceae family (Graham, 1964; Bartish and Swenson, 2004), have white, creamy-white, or light yellow perianth (Figure 4). The flower tube is tubular or funnel-cylindrical, four-lobed, 4.0–5.5 mm in diameter and 5–10 mm long, narrowed above the ovary, breaks off as the fruit develops; blades are broadly ovate, sharply pointed at the top. Stamens 4, alternate with calyx lobes; the threads are very short. The ovary is upper with one seed primordium. Style is oblong; stigma is dry. Pedicel 4–8 mm long; during fruiting, it lengthens to 1.5–5.0 cm, thin.

From two to seven to eight flowers are usually formed on one shoot, depending on the genotype and weather conditions (Figure 5). Flowering begins in late April or early May and lasts from 15 to 20 days. The duration

Figure 5 Flowering shoots of *Elaeagnus multiflora* Thunb. plants

of flowering depends both on the genotype and on weather conditions, in particular on temperature and amount of precipitation. The sum of effective temperatures at the beginning of flowering during 1999–2003 was 204.5–225.5 °C (Vasyuk and Moroz, 2005).

Principal growth stage 7: Fruit development

Fruits are propagules for the reproduction and dispersal of angiosperms and at the same time are products of many agricultural crops. Fleshy fruits play a particularly important role in a person's daily diet. The edible, juicy part of such fruits has very diverse origin. Almost all parts of the entire structure of the inflorescence can form fruit pulp in certain species (Coombe, 1976).

The fruits of *E. multiflora* are false drupes (sphalerocarps), as in other species of the Elaeagnaceae family. They are covered with a juicy hypanthium base (calyx tubes). Only the abaxial layers of hypanthium cells form the pulp, while the adaxial ones are transformed into mechanical tissue such as an eight-ribbed "stone" (Qin and Gilbert, 2007; Ye et al., 2012). The fruit (pericarp) is thin, membranous, and consists of several layers of cells, that is why cherry elaeagnus fruits are classified as false fruits namely stone-like or berry-like (Graham, 1964; Bartish and Swenson, 2004).

E. multiflora fruits develop and grow quite quickly. About three to five weeks pass from the beginning of the growth of the ovaries (phenophase 71) to reaching the final size of the first fruits (phenophase 79). Ovaries and immature fruits that have finished growing are hard, green, and abundantly covered with brown peltate scales. The rapid development of fruits is also characteristic of the Japanese variation of *E. multiflora* var. *gigantea* Araki, in which the period from flowering to fruit ripening is 6–7 weeks (Ye et al., 2012).

Principal growth stage 8: Maturity of fruit

Ripe *E. multiflora* fruits are elongated, ovoid, or ellipsoidal false drupes with a length of 7.60 to 19.54 mm and a diameter of 4.39 to 10.32 mm (Grygorieva et al., 2018b). The beginning of fruit ripening starts with a change in their color (stage 81). They initially become yellow, and then gradually acquire a red color, characteristic of the studied *E. multiflora* genotypes. Ripe fruits are red with silver or brown scales, have thin long peduncles (up to 5 cm long) (Figure 6, 7), remain on the plant for a long time, and do not fall off. On the same shoot, as well as on the same tree, the fruits ripen at different times, which corresponds to the gradual development of generative buds. The fruit ripening period begins in the first or second decade of June at the sum of effective temperatures of 616.6–790.2 °C (Vasyuk and Moroz, 2005) and lasts about two to three weeks. For use, the fruits are collected at the stage of technical ripeness (phenophase 87).

E. multiflora has abundant and regular fruiting (Figure 8). Ripe fruits are sweet with a slight astringency. The skin of the fruit is thin and fragile, which determines the specificity of the use of the species as a food crop. *E. multiflora* begins to bear fruit in the 4^{th}–5^{th} year. The most productive fruiting occurs at the age of 8 and lasts at least 12–15 years.

Figure 6 Fruits and seeds of *Elaeagnus multiflora* Thunb.

Figure 7 Fruits and peduncle of *Elaeagnus multiflora* Thunb.

Figure 8 Fruiting of *Elaeagnus multiflora* Thunb. plants

Principal Growth Stage 9: Senescence and beginning of the rest period

The stage of senescence and the onset of dormancy in the studied *E. multiflora* genotypes is extended in time, as in other fruit plants of a temperate climate (Martínez-Valero et al., 2001, 2019; Grygorieva et al., 2010, 2018; Atay, 2013; Klymenko and Ilyinska, 2021; Klymenko et al., 2021). First, the oldest leaves change color and fall (phenophases 92, 93), and later others die. Under optimal weather conditions, November (phenophases 93, 95) in the studied *E. multiflora* genotypes is quite long and not intense. Rapidly, within several days, the fall of leaves can be caused by a significant night frost. All leaves (phenophases 97) shed infrequently. Usually, one or more leaves can remain on the tops of annual shoots for a very long time.

Conclusions

In the climatic conditions of Ukraine (NBG, Kyiv), the studied genotypes of *E. multiflora* go through a full cycle of development. Plants begin their growing season with the development of leaves and shoots. According to the international BBCH scale, they clearly distinguish seven of the ten main stages of seasonal development, in particular: the development of buds (Principal growth stage 0), leaves (Principal growth stage 1), shoots (Principal growth stage 3), flowering (Principal growth stage 6), fruit development (Principal growth stage 7), fruit ripening (Principal growth stage 8) and senescence and onset of dormancy (Principal growth stage 9). Understanding the phenology of *E. multiflora* as a rare, but promising medicinal plant of Ukraine, namely the codification of seasonal stages of growth, according to the international BBCH scale, is important for the evaluation of breeding material and breeding varieties, improving the technological qualities of fruits. The proposed BBCH scale for characterizing the stages of seasonal development of *E. multiflora* plants is important for further research on the adaptive capabilities of the species under different climatic conditions, for the practical use of the complex of gumi genetic resources, as well as for its introduction and use in agricultural production, pharmacology, decorative and landscape gardening.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

Funding

This work was supported by the grant Visegrad Fund and SAIA (Slovakian National Scholarship Program) for the scholarship for the research during which the presented knowledge was obtained.

Acknowledgements

The publication was prepared with the active participation of researchers in the International Network AgroBio*Net*.

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

OPEN ACCESS

Oxidative stress biomarkers in equine erythrocytes and plasma after *in vitro* treatment with an aqueous leaf extract of *Coelogyne brachyptera* Rchb. f. (Orchidaceae)

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Article Details:
Received: 2022-07-30
Accepted: 2022-08-29
Available online: 2022-11-30

DOI: https://doi.org/10.15414/ainhlq.2022.0025

Many orchids have been used as medicinal plants for many years in China, Japan, India, and some other countries. They showed many health-beneficial functions, such as the protection of cells against free radicals and oxidative stress possessing hepatoprotection, cardioprotection, gastroprotection, neuroprotection, and other properties. This study aimed to investigate the oxidative stress biomarkers [2-thiobarbituric acid reactive substances (TBARS), aldehydic and ketonic derivatives of oxidatively modified proteins (OMP), and total antioxidant capacity (TAC)] in the equine erythrocytes and plasma after *in vitro* incubation with an extract derived from leaves of *Coelogyne brachyptera* Rchb. f. The leaves of *C. brachyptera* plants, cultivated under glasshouse conditions, were sampled at M.M. Gryshko National Botanic Garden (NBG), National Academy of Science of Ukraine. Freshly collected leaves were washed, weighed, crushed, and homogenized in 0.1M phosphate buffer (pH 7.4) (in the proportion of 1:19, w/w). The equine plasma and erythrocyte aliquots were used in the study. The pellet of blood was re-suspended in phosphate buffer (pH 7.4). A volume of 0.1 ml of the *C. brachyptera* extract was added to 1.9 ml of clean equine erythrocytes or 1.9 mL of plasma. For positive control (blank), 0.1M phosphate buffer (pH 7.4) added to erythrocytes or plasma was used. Results of our study revealed that erythrocytes were more sensitive to the action of an extract derived from leaves of *C. brachyptera*. The levels of aldehydic and ketonic derivatives of oxidatively modified proteins in the treated erythrocytes were significantly decreased, while these parameters were no-changed in the equine plasma. The treatment of equine erythrocytes by extract derived from leaves of *C. brachyptera* increased lipid peroxidation. On the other hand, plasma TBARS level after treatment by extract derived from leaves of *C. brachyptera* was at the same level as in untreated controls. The level of total antioxidant capacity was not-significantly changed after treatment both in equine plasma and erythrocytes. Studies concerning the antioxidant properties of orchids are continued in our laboratory.

Keywords: leaf extract, equine erythrocytes and plasma, lipid peroxidation, oxidatively modified proteins, total antioxidant capacity

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Introduction

Orchidaceae is one of the largest and more diverse families of flowering plants with approximately 25,000 species in 736 genera currently recognized, as well as widely distributed as epiphytes, lithophytes, or terrestrials (Chase et al., 2015). Orchids have been used all over the world in traditional healing and treatment systems for various diseases, such as chest pain, arthritis, syphilis, jaundice, cholera, acidity, eczema, tumor, piles, tuberculosis, wounds, stomach disorders, boils, inflammation, menstrual disorders, spermatorrhea, leucoderma, slantendicular, muscular pain, earache, sexually transmitted diseases, blood dysentery, hepatitis, bone fractures, rheumatism, asthma, malaria, paralysis, and dyspepsia (Kong et al., 2003; Pant, 2013; Rahman et al., 2022). It is suggested that the pharmaceutical properties of orchids are due to the activities of many phytochemicals, including alkaloids, bibenzyl derivatives, flavonoids, carotenoids, phenanthrenes, phenanthropyrans, stilbenes, anthocyanins, glycosides, sterols, and terpenoids, which are present in various parts of orchid plants (Zhang et al., 2015; Axiotis et al., 2021). In recent years, the assessment of the anti-diabetic, anti-inflammatory, and antioxidant properties of orchids has received considerable attention (Li et al., 2018; Warinhomhoun et al., 2021; Zhang et al., 2021). Some orchid species are used as a potent antioxidant and cytotoxic activities and also proved to be potent antioxidant agents (Paudel et al., 2018, 2019; Robustelli Della Cuna et al., 2019; Li et al., 2021).

Previously, we have given considerable attention to the evaluation of the antibacterial activity of ethanolic extracts derived from leaves and pseudobulbs of plants belonging to various *Coelogyne* species, maintained under glasshouse conditions (Buyun et al., 2016-2019a,b, 2021). For example, the assessment of the antifungal potential of orchids species, i.e. *Coelogyne cristata* Lindl., *C. fimbriata* Lindl., *C. flaccida* Lindl., *C. huettneriana* Rchb.f., *C. ovalis* Lindl., *C. speciosa* (Blume) Lindl., *C. tomentosa* Lindl. and *C. viscosa* Lindl. against fungus strain, *Candida albicans* was conducted by Buyun et al. (2018). Marked antifungal efficacy was observed in the case of ethanolic extracts derived from leaves of *C. flaccida* (mean diameter of inhibition zones was 19.5 mm), *C. viscosa* (18.6 mm), *C. huettneriana* (18.2 mm), and *C. fimbriata* (17.5 mm). Extracts of *C. cristata*, *C. ovalis*, and *C. tomentosa* displayed less profound inhibitory activity against test fungus (mean diameter of inhibition zones ranging from 16 to 17.5 mm). Similarly, the ethanolic extracts from the pseudobulbs of eight *Coelogyne* species exhibited strong activity against *C. albicans* (inhibition zone diameter ranged from 16 to 23.5 mm). Moreover, it has been observed that ethanolic extract from pseudobulbs of *C. speciosa* revealed the highest antibacterial activity (21 mm as the diameter of the inhibition zone) among various *Coelogyne* species screened. The results also indicate that scientific studies carried out on medicinal plants having traditional claims of effectiveness might warrant fruitful results (Buyun et al., 2018).

Later, we also assessed the oxidative stress biomarkers (2-thiobarbituric acid reactive substances (TBARS), aldehydic and ketonic derivatives of oxidatively modified proteins (OMP), total antioxidant capacity (TAC)) in the equine erythrocytes and plasma after treatment *in vitro* by extract derived from leaves of *Dendrobium parishii* Rchb. F. (Buyun et al., 2019b). The levels of TBARS as a biomarker of lipid peroxidation, aldehydic and ketonic derivatives of OMP, as well as TAC, were non-significantly altered in the erythrocyte suspension after *in vitro* incubation with an extract derived from leaves of *D. parishii*. More significant changes were observed in the equine plasma. The *D. parishii* extract caused to increase in the formation of intracellular aldehydic and ketonic derivatives of OMP in the extract-treated plasma, but these results were non-significant. Total antioxidant capacity was non-significant decreased both in plasma and erythrocytes (Buyun et al., 2019b).

The current study is a continuation of our cooperation with M.M. Gryshko National Botanic Garden, National Academy of Science of Ukraine (Kyiv, Ukraine) concerning investigations of antibacterial and antioxidant properties of extracts derived from leaves and pseudobulbs of some species belonging to the Orchidaceae family using different cell models *in vitro*. We have chosen equine erythrocytes as a model for the evaluation of the antioxidant properties of plant extract because equine erythrocytes are more sensitive to oxidant-induced damage due to the use of inefficient mechanisms to correct and protect against oxidative damage, i.e. methemoglobin formation, alteration of aggregation, and reduction of cellular deformability (Baskurt and Meiselman, 1999). Equine erythrocytes are slower than erythrocytes from other species studied in their ability to regenerate glutathione (GSH) after it has been oxidized *in vitro* (Harvey et al., 2003). Moreover, sulfhydryl groups in proteins and unsaturated lipids in membranes are especially susceptible to oxidation. Oxidative denaturation and the precipitation of the globin portion of hemoglobin into large aggregates result in the formation of Heinz bodies that can bind to and alter membranes.

Membrane structure also is altered by the oxidation of sulfhydryl groups and by lipid peroxidation (Harvey, 1997).

Thus, this study aimed to investigate the oxidative stress biomarkers (2-thiobarbituric acid reactive substances, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity) in the equine erythrocytes and plasma after *in vitro* incubation with an extract derived from leaves of *Coelogyne brachyptera* Rchb. f.

Material and methodology

Collection of plant materials

The leaves of *C. brachyptera* plants cultivated under glasshouse conditions were sampled at M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine (NBG, Kyiv, Ukraine) in September 2016 (Figure 1). Since 1999, the whole collection of tropical and subtropical plants (including orchids) has had the status of a National Heritage Collection of Ukraine and is supported through State Funding. Besides, the NBG collection of tropical orchids was registered at the Administrative Organ of CITES in Ukraine (Ministry of Environment Protection, registration No. 6939/19/1-10 of 23 June 2004).

Various databases are available for searching collections of living plants, confirming the taxonomic identity of having been reviewed, e.g. World Checklist of Orchidaceae (Govaerts et al., 2016), International Plant Names Index, The Plant List, the IUCN Red List (IUCN, 2013).

Coelogyne brachyptera is found in Burma, Thailand, Cambodia, Laos, and Vietnam. It grows epiphytically in the primary mountain forest, the most frequent at an altitude of 1,000 to 2,500 meters above sea level

Figure 1 Vegetative shoot with inflorescence of *Coelogyne brachyptera* Rchb. f. plant, cultivated at NBG's glasshouses (Kyiv, Ukraine)
Photo: Oleksandr Gyrenko

(Averyanov et al., 2003). It is a sympodial orchid with pseudobulbs of one internode, narrowly conical, 4-angled, slightly grooved, pale green, carrying 2 leaves. The leaves are elliptic to elliptic-lanceolate, subacute, plicate, 7-nerved, with an undulate margin. The flowering of *C. brachyptera* under glasshouse conditions at NBG was observed in March – April. The duration of anthesis of a single inflorescence did not exceed 2 weeks.

Preparation of plant extracts

Freshly collected leaves were washed, weighed, crushed, and homogenized in 0.1 M phosphate buffer (pH 7.4) (in the proportion of 1:19, w/w) at room temperature. The extracts were then filtered and used for analysis. All extracts were stored at -25 °C until use. All biochemical assays were conducted at the Department of Biology, Institute of Biology and Earth Sciences, Pomeranian University in Słupsk (Poland).

Horses

Eighteen clinically healthy adult horses from the central Pomeranian region in the northern part of Poland (Strzelinko village, N 54º 30' 48.0" E 16º 57' 44.9"), aged 8.9 ±1.3 years old, including 6 Hucul ponies, 5 Thoroughbred horses, 2 Anglo-Arabian horses and 5 horses of unknown breed, were used in this study. All horses participated in recreational horseback riding. Horses were housed in individual boxes, with feeding (hay and oat) provided twice a day, at 08.00 and 18.00 h, and water available *ad libitum*. Before sampling, all horses were thoroughly examined clinically by a veterinarian and screened for hematological, biochemical, and vital parameters, which were within reference ranges. The females were non-pregnant.

Collection of blood samples

Blood samples were collected in the morning, 90 minutes after feeding, while the horses were in the stables (between 8:30 and 10 AM) by jugular venipuncture into tubes with sodium citrate as the anticoagulant and held on the ice until centrifugation at 3,000 rpm for 5 min to remove plasma Blood was stored into The pellet of blood was re-suspended in 4 mM phosphate buffer (pH 7.4). A volume of 0.1 ml of the plant extract was added to 1.9 ml of equine erythrocytes or plasma. For positive control, 0.1 ml of phosphate buffer added to the equine erythrocytes or plasma was used. After incubation of the mixture at 37 °C for 60 min with continuous stirring, biochemical assays were done. Erythrocytes and plasma aliquots were used in the study.

The 2-Thiobarbituric acid reactive substances (TBARS) assay

The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reacting substances (TBARS) with the Kamyshnikov (2004) method for determining the malonic dialdehyde (MDA) concentration. This method is based on the reaction of the degradation of the lipid peroxidation product, MDA, with 2-thiobarbituric acid (TBA) under high temperature and acidity to generate a colored adduct that is measured spectrophotometrically. The nmol per 1 mL was calculated using $1.56 \cdot 10^5$ $mM^{-1}.cm^{-1}$ as the extinction coefficient.

The carbonyl derivatives of oxidative modification of proteins (OMP)

To evaluate the protective effects of the extract against free radical-induced protein damage in equine erythrocytes and plasma, carbonyl derivatives of protein oxidative modification (OMP) assay based on the spectrophotometric measurement of aldehydic and ketonic derivatives in the samples was performed. The rate of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives of amino acid reaction with 2,4-dinitrophenylhydrazine (DNFH) as described by Levine et al. (1990) and as modified by Dubinina et al. (1995). DNFH was used for determining carbonyl derivatives in soluble and insoluble proteins. Carbonyl groups were determined spectrophotometrically from the difference in absorbance at 370 nm (aldehydic derivatives, OMP_{370}) and 430 nm (ketonic derivatives, OMP_{430}).

Measurement of total antioxidant capacity (TAC)

The TAC level in samples was estimated by measuring the 2-thiobarbituric acid reactive substances (TBARS) level after Tween 80 oxidation. This level was determined spectrophotometrically at 532 nm (Galaktionova et al., 1998). The sample inhibits the Fe^{2+}/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. The level of TAC in the sample (%) was calculated concerning the absorbance of the blank sample.

Statistical analysis

The mean ± S.E.M. values were calculated for each group to determine the significance of the intergroup difference. All variables were tested for

normal distribution using the Kolmogorov-Smirnov and Lilliefors test ($p > 0.05$). The significance of differences between the total antioxidant capacity level (significance level, $p < 0.05$) was examined using the Mann-Whitney *U* test (Zar, 1999). In addition, the relationships between oxidative stress biomarkers were evaluated using Spearman's correlation analysis. All statistical calculations were performed on separate data from each individual with Statistica 13.3 software (TIBCO Software Inc., Krakow, Poland).

Results and discussion

The TBARS content as a biomarker of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins, and the total antioxidant capacity (TAC) in the equine erythrocytes after *in vitro* incubation with an extract derived from leaves of *C. brachyptera* was assessed and shown in Figure 2.

Lipid peroxidation can be broadly defined as the process of inserting a hydroperoxy group into a lipid. Polyunsaturated fatty acids present in the phospholipids, indispensable for the normal structure of membranes, are often the targets for peroxidation (Anthonymuthu et al., 2016). The 2-thiobarbituric acid reactive substances (TBARS) assay has been widely used as a generic metric of lipid peroxidation in biological fluids (Aguilar Diaz De Leon et al., 2020). As presented in Figure 2, treatment by extract derived from leaves

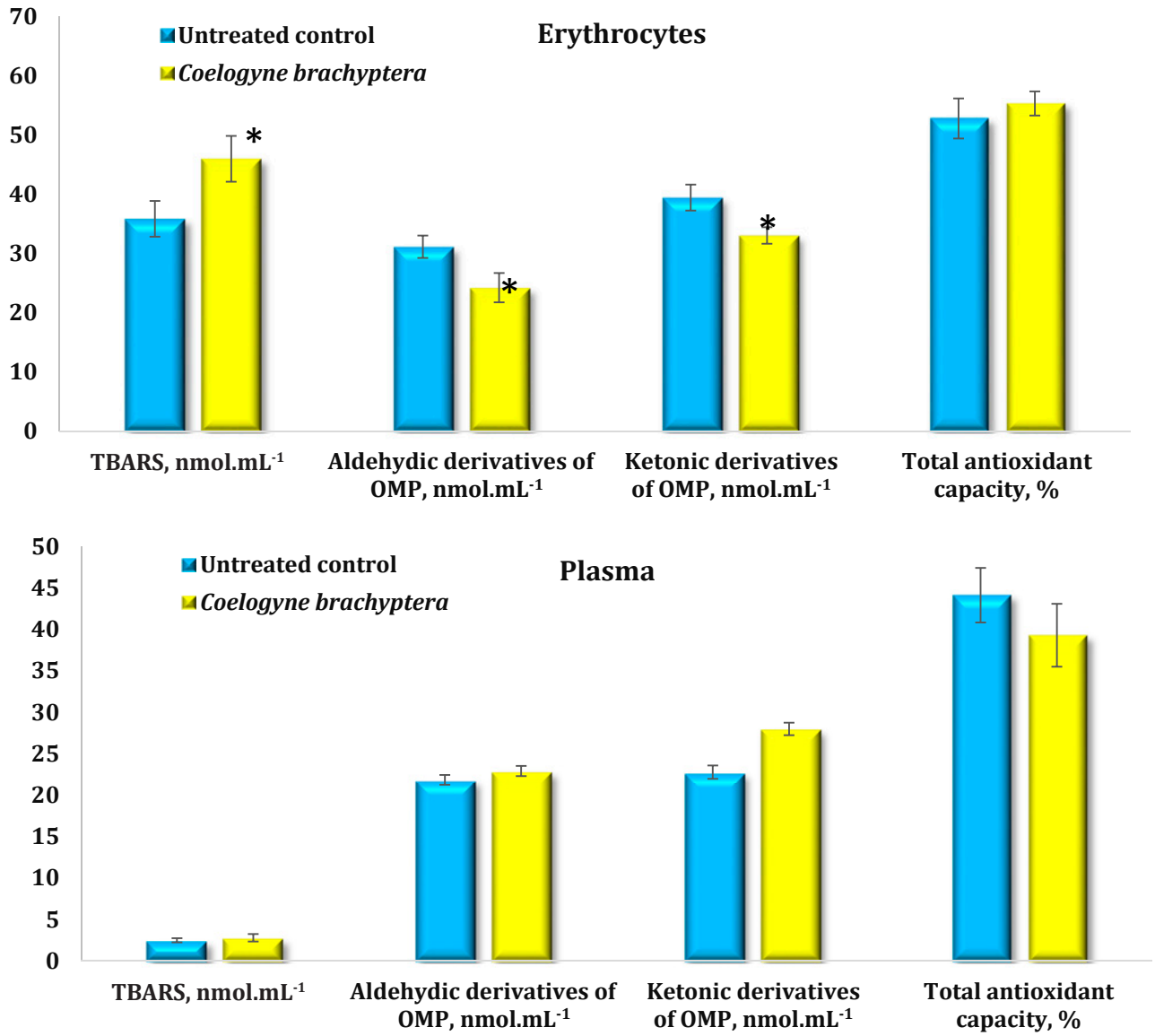

Figure 2 The TBARS content as a biomarker of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity in the equine erythrocytes and plasma after *in vitro* incubation with an extract - of *Coelogyne brachyptera* Rchb. f. (M ±m, n = 18)
*– statistically significant differences between treated and untreated samples ($p < 0.05$)

of *C. brachyptera* resulted in an increase in erythrocyte TBARS level of (46.02 ±3.86 nmol.mL^{-1}) compared to the untreated samples (35.88 ±3.02 nmol.mL^{-1}). The increase in TBARS level was by 28.3% (p = 0.005). On the other hand, plasma TBARS level after treatment by extract derived from leaves of *C. brachyptera* was at the same level as in untreated controls (2.80 ±0.45 nmol. mL^{-1} vs. 2.49 ±0.25 nmol.mL^{-1}) (Figure 2).

Proteins are major targets for oxidative stress because they are abundant and have rapid rates of reaction with a wide range of radicals and excited state species. Exposure of proteins to free radicals resulted in the loss of the parent amino acid residue, the formation of unstable intermediates, and the generation of stable products (Hawkins et al., 2009; Hawkins and Davies, 2019). The levels of aldehydic and ketonic derivatives of oxidatively modified proteins were also decreased in samples treated with an extract derived from leaves of *C. brachyptera* compared to the untreated samples, and these decreases were statistically significant (p <0.05). When equine erythrocytes were incubated with the extract derived from leaves of *C. brachyptera*, the levels of aldehydic and ketonic derivatives were significantly decreased by 22.1% (24.26 ±2.48 nmol.mL^{-1} vs. 31.16 ±1.89 nmol.mL^{-1}) and 16.2% (p <0.05) (33.07 ±1.40 nmol.mL^{-1} vs. 39.47 ±2.20 nmol.mL^{-1}). On the other hand, aldehydic derivatives of oxidatively modified proteins in the equine plasma after treatment by an extract derived from leaves of *C. brachyptera* were at the same levels as untreated samples (22.91 ±0.62 nmol.mL^{-1} vs. 21.85 ±0.59 nmol.mL^{-1}). Ketonic derivatives of oxidatively modified proteins in the equine plasma after treatment by an extract derived from leaves of *C. brachyptera* were a statistically non-significant increase by 22.9% (p >0.05) (27.99 ±0.75 nmol.mL^{-1} vs. 22.77 ±0.80 nmol.mL^{-1}) (Figure 2). Also, a non-significantly increase in erythrocyte TAC level was observed after incubation with an extract derived from leaves of *C. brachyptera* (by 4.8%, p >0.05) (55.37 ±2.04% vs. 52.83 ±3.38%) (Figure 2). TAC levels in the equine plasma after treatment by an extract derived from leaves of *C. brachyptera* were a statistically non-significant decrease by 11% (p >0.05) (39.28 ±3.79% vs. 44.11 ±3.29%) (Figure 2).

The efficacy of some plants belonging to the *Coelogyne* genus was reported by some researchers using *in vitro* and *in vivo* models. For example, Sharma et al. (2014) have evaluated the bone-forming activity of *Coelogyne cristata* Lindl. extract *in vivo* wherein parameters like trabecular microarchitecture, bone strength, and uterine estrogenicity were studied in the estrogen-deficient female Balb/c mice model. Subsequently, coelogin, a pure compound isolated from ethyl acetate fraction of *C. cristata* alcoholic extract was evaluated in *in vitro* osteoblast cell cultures, alkaline phosphatase activity (a marker of osteoblast differentiation), mineral nodule formation and mRNA levels of osteogenic markers like BMP-2, Type 1 Collagen, and RUNX-2. Their experimental results suggest that both ethanolic extract and Coelogin isolated from *C. cristata* possess significant improvement of trabecular response leading to the restoration of trabecular microarchitecture in both femoral and tibial bones in ovariectomized estrogen-deficient mice along with the biochemical strength. The study by Sharma et al. (2014) also supports the use of *C. cristata* for the treatment of fracture healing as claimed by traditional practitioners. The identified bioactive compound may serve as the starting point for the design and development of pharmaceutical products not only to reduce fracture risk but also for the management of postmenopausal osteoporosis (Sharma et al., 2014). The osteoprotective effect of coelogin was also evaluated on osteopenic adult female Swiss mice in the study by Prakash et al. (2021). Coelogin treatment led to increased osteoblast proliferation, survival, differentiation, and mineralization in osteoblast cells. Coelogin supplementation to Ovx mice promoted new bone formation, prevented Ovx-induced deterioration of bone microarchitecture, and enhanced bone regeneration. In addition, signaling studies revealed that coelogin treatment activates the ER-Erk and Akt-dependent signaling pathways which stimulate osteoblastogenesis in osteoblast cells (Prakash et al., 2021).

The effectiveness of the phenanthrene-rich hydro-alcoholic extract of pseudobulbs of *C. cristata* (CCE) in chronic fatigue syndrome (CFS)-induced behavioral changes in aged animals was done by Mitra et al. (2018). Biochemical estimations were also carried out to establish the antioxidant activity of this plant *in vivo* whereas *Panax ginseng* C.A. Mey. was used as the prototype standard. CCE was found to be non-toxic. CCE-treated aged rats significantly improved the spontaneous locomotor movement concerning control rats, while, decreasing the mobility period or depression score. In CFS, CCE also enhanced the time spent in open arms while reducing the time spent in closed arms as compared to CFS control, indicating lowering anxiety score. Moreover, a marked diminution in lipid peroxidation, nitrite, and superoxide dismutase levels were exhibited after CCE treatment and significantly enhanced catalase level significantly concerning CFS control. *Panax ginseng* also showed similar actions. The results of Mitra et al. (2018) confirmed the potential

therapeutic actions of CCE against experimentally induced CFS in aged rats that might be due to its CNS mediatory antioxidant properties.

Antioxidant and antitumor properties also were revealed for other orchids. For example, data obtained by Zhao et al. (2019) introduces the novel idea that *Dendrobium officinale* Kimura & Migo polysaccharides (DOP) prevent precancerous lesions of gastric cancer (PLGC). These authors demonstrated that DOP are capable of restraining the activity of 8-hydroxy-deoxyguanosine while increasing the nuclear expression of nuclear factor erythroid 2-related factor 2. All told, this activates downstream heme oxygenase-1 and NADPH quinone oxidoreductase-1 expression to improve antioxidant activity and protect gastric mucosal cells from oxidative damage. In addition, DOP can decrease serum levels of alanine aminotransferase, serum uric acid, and blood urea nitrogen, indicating DOP might protect liver and kidney function. These findings show DOP can be considered an effective healthcare product for the treatment of precancerous lesions of gastric cancer (Zhao et al., 2019).

Thus, in the current study, we have undertaken an attempt to investigate the *in vitro* antioxidant activity of an extract derived from the leaves of *C. brachyptera* plants using as a model equine erythrocytes and plasma. The results obtained suggested that an extract derived from the leaves of *C. brachyptera* significantly decrease the protein oxidation in the erythrocyte suspension after *in vitro* treatment. On the other hand, lipid peroxidation was enhanced. In the equine plasma, an extract derived from the leaves of *C. brachyptera* resulted in non-significant alterations in levels of lipid peroxidation, oxidatively modified proteins, and TAC.

Conclusions

In the current study, we investigated the changes in the oxidative stress biomarkers using the model of equine erythrocytes and plasma to evaluate the antioxidant activities of the aqueous extract derived from leaves of *C. brachyptera*. Results of our study revealed that erythrocytes were more sensitive to the action of an extract derived from leaves of *C. brachyptera*. The levels of aldehydic and ketonic derivatives of oxidatively modified proteins in the treated erythrocytes were significantly decreased, while these parameters were no-changed in the equine plasma. The treatment of equine erythrocytes by extract derived from leaves of *C. brachyptera* increased lipid peroxidation. On the other hand, plasma TBARS level after treatment by extract derived from leaves of *C. brachyptera* was at the same level as in untreated controls. The level of total antioxidant capacity was not-significantly changed after treatment both in equine plasma and erythrocytes. Studies concerning the antioxidant properties of orchids have continued in our laboratory. The next step in our further investigation will be HPLC profiling of the plant extract to find new bioactive compounds from a natural source.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

Funding

This work was supported by The International Visegrad Fund, and the authors are cordially grateful for this.

Acknowledgement

This research was supported by the Pomeranian University in Słupsk (Poland).

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

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Morphological characteristics of selected fruit parts and naked seeds of *Cucurbita pepo* var. *styriaca*

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Article Details:
Received: 2022-08-01
Accepted: 2022-09-02
Available online: 2022-11-30

DOI: https://doi.org/10.15414/ainhlq.2022.0026

The study aimed to determine the variability of some morphological characters on fruits of selected plants within the population of ESO variety of Styrian oil pumpkin (*Cucurbita pepo* var. *styriaca*). For experimental evaluation, we used two years (2020–2021) measurements of 165 selected fruits from individual plants grown in the field conditions in Kolíňany (Slovak Republic). We determined for all samples the range for the weight of fruits (521.50–12744.70 g), length of fruits (85.00–285.00 mm), width of fruits (114.00–320.00 mm), exocarp thickness on the left side (0.10–4.86 mm), exocarp thickness on the right side (0.33–4.56 mm), pericarp thickness on the left side (9.13–57.02 mm), pericarp thickness on the right side (9.77–46.05 mm), placenta weight (89.00–870.00 g), fresh seed weight (17.30–638.50 g), seed weight after drying (4.53–187.36 g), seed length (12.91–24.00 mm), seed diameter (6.56–14.41 mm), seed thickness (0.41–3.62 mm), the weight of one thousand seeds (15.41–475.66 g). The results document that in our collections there are plants suitable for the next cultivation, and further selective improvement. In practice, only seeds of the ESO variety are used for oil production. The percentage of the weight of the seeds from the total weight of the fruits is only 3–4%. Other parts of the fruit remain in the field after harvesting. After their decomposition and ploughing into the soil, they improve the physico-chemical properties of the soil. However, the results of the experiments confirm that many parts of the fruit, such as the pulp and the placenta, are important raw material sources for use in the food industry, pharmaceuticals, cosmetics, and other areas. When growing butter pumpkin in large areas, it is also possible to collect and use flowers and young – unripe fruits from the plants during the growing season in the food industry and other areas.

Keywords: Styrian oil pumpkin, varieties, morphometric analysis, variability

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Introduction

Cucurbita spp. (pumpkins) is collectively ranked among the 10 leading vegetable crops worldwide. China and India are the world's leading producers. Other major producers are the U.S.A, Egypt, Mexico, Ukraine, Cuba, Italy, Iran and Turkey (FAOSTAT, 2013). *Cucurbita* spp. are members of the economically important Cucurbitaceae Juss. This family is also called "Cucurbits" popularly known as the "Gourd family". There are three economically important *Cucurbita* species, namely *Cucurbita pepo* L., *Cucurbita maxima* Duch. and *Cucurbita moschata* Duch., which have different climatic adaptations and are widely distributed in agricultural regions worldwide (Robinson and Decker-Walters, 1997; Paris and Brown, 2005; Wu et al., 2007; Balkaya et al., 2010). These species belong to the melon family Cucurbitaceae which is a diverse class of plants that consists of at least 8 tribes, 119 genera and over 825 species (OECD, 2016; Andres, 2003).

Domesticated *Cucurbita* has been remarked as one of the plant genera with the highest diversity in colour, shape and fruit dimensions. Five species in decreasing order of importance are now cultivated worldwide, i.e., *C. pepo*, *C. moschata*, *C. maxima*, *C. argyrosperma* and *C. ficifolia*, and, in production, they are positioned among the ten most important world vegetable crops (Esquinas- Alcazar and Gulick, 1983; Smith, 2006; Ferriol and Picó, 2008).

Cucurbita pepo is indigenous to warm and temperate regions of Central and North America and is cultivated there. It also exists in the wild form in Europe and Asia. The origin is uncertain. The common ancestor of all the current *Cucurbita pepo* varieties originates probably from Mexico as confirmed by archaeological findings (King, 1985; Paris, 1989).

Cucurbita pepo subsp. *pepo* var. *styriaca* is a phylogenetically young member of the *Cucurbita* spp. whereas arose only in the 19th century in Austria's south-eastern province Styria. This mutation defined the so-called Styrian oil pumpkin and facilitated the production of Styrian pumpkin seed oil. This pumpkin variety is called "Styrian oil pumpkin" and has been formed by an accidental natural mutation that led to tremendous morphological changes in the seed architecture. This mutation, which is the result of a single recessive gene (Stuart, 1983), led to a very thin outer hull (naked or hull-less seeds), which highly facilitates the production of this regional speciality oil and also leads toits dark green colour. In these seeds, the amounts of lignin and cellulose in the hypodermis, sclerenchyma and parenchyma tissues of the seed coat are reduced (Loy, 2000, 2004).

Naked pumpkin seeds are a popular ingredient in many snacks, bread, breakfast cereals, soups, and other edible goods (Loy, 2004; Baxter et al., 2012). Vegetable oil derived from the seeds can be purchased by the bottle for culinary/condiment use or as capsules in health food stores (Stevenson et al., 2007). Unlike conventional hulled pumpkin seeds, naked seeds lack a complete seed coat and thus are preferred for snacking and oil production because they eliminate the need for manual dehulling prior to use.

Pumpkin seeds are rich in oil (50% w/w), protein (35%), unsaturated fatty acids (86%) (Meru et al., 2018), and antioxidants that have many health benefits, including a reduced risk of certain cancers (Nesaretnam et al., 2007; Stevenson et al., 2007; Lelley et al., 2009), treatment of enlarged prostate, and lowering cholesterol levels (Thompson and Grundy, 2005; Fruhwirth and Hermetter, 2007).

Styrian pumpkin seed oil is also of considerable economic importance for the province of Styria. The Styrian oil pumpkin is the third most important field fruit in Styria with 13,000 ha of cultivable land yielding 11,100 tons in the year 2006, as reported Fruhwirth and Hermetter (2007). The average yield of pumpkin seeds of this variety strongly depends on the weather conditions, ranging from approximately 400 kg.ha^{-1} (under drought) up to 1000 kg.ha^{-1} under optimal conditions, with an average yield of 500–600 kg.ha^{-1}. To produce 1 L of this speciality oil, an average of 2.5 kg of pumpkin seeds is required, which corresponds to an amount of 30–40 oil pumpkins. However, this value is heavily influenced by cultural conditions and by the breeding line itself (FAOSTAT, 2013).

Data on phytochemistry and pharmacological activity of reported *Cucurbita pepo* chemical constituents extracted, partly used, and types of pharmacological activity performed so far. These activities of *C. pepo* might be due to the existence of certain classes of compounds including flavonoids, terpenoids, cardiac glycosides and cucurbitacins glycoside. *C. pepo* is also rich in nutritional components like carbohydrates, proteins, lipids and minerals (Adnan et al., 2017). Such differences may be caused by variations in cultivar or origin (Tsaknis et al., 1997). The content of amino acids, fatty acids and minerals may vary considerably depending on different conditions (Glew et al., 2006).

Cucurbita pepo is widely used like food and in folk medicine around the world (Perez-Gutierrez, 2016).

This aim is comprehensive of the pharmacological, chemical constituents, and clinical uses. Also have been identified the medicinally important phytoconstituents belonging mainly to cucurbitosides, multiflorane-type triterpenoids, carotenoids, ent-kaurane-type diterpene, and cucurbita glycosides. Extracts and metabolites of this plant, particularly those from seeds and fruits possess useful pharmacological activities. A survey of the literature shows *C. pepo*, is mainly known for its improvement in prostatic hyperplasia (Abdel-Rahman, 2006; Gossell-Williams et al., 2006), urinary dysfunction and cytotoxic properties (Bombardelli and Morazoni, 1997), also been used extensively as a hypoglycaemic agent (Zhang, 2004; Quanhong et al., 2005; Sesti, 2006). Many pharmacological studies have demonstrated hepatoprotection (Nkosi et al., 2006a,b), inhibit benign prostatic hyperplasia (Gossell-Williams et al., 2006), antioxidant (Nawirska-Olszanska et al., 2013; Song et al., 2015), anticancer (Matus et al., 1993), antimicrobial (Dubey et al., 2010; Noumedem et al., 2013), anti-inflammatory (Park et al., 2004), antidiabetic (Sesti, 2006; Bharti et al., 2013), antiparasitic (Jiang and Du, 2011), and antiulcer activities supporting its traditional uses (Gill and Bali, 2011).

Styrian oil pumpkin has become the object of study in many countries of the world. In general, research focuses on the morphological characteristics of characters on fruits and flowers, chemical composition, antioxidant activity (Brindza et al., 2011, 2014; Muntean et al., 2012; Oleárová et al., 2013), conservation of genetic resources (Mendel et al., 2019; Avagyan et al., 2020) and other possibilities of practical use. Pumpkin also produces a lot of pollen, which can be collected by bees in the form of bee pollens (Chlebo et al., 2017).

The experiment aimed to determine the production and variability of some morphological characters on fruits taken from selected individual plants within the ESO variety of oil pumpkin (*Cucurbita pepo* var. *styriaca*).

Material and methodology

Biological material

In the experiments, 165 fruits from randomly selected plants from the cultivated population of *Cucurbita pepo* var. *styriaca* on an area of 150 ha were evaluated. Fruits were taken in September and October 2020, and 2021 and analysed in the morphometric laboratory at the Institute of Plant and Environmental Sciences in Nitra (Slovak Republic).

Morphometrical analysis

The following characters were evaluated:

A total of 10 quantitative characters were evaluated in the fruits and 4 quantitative characters in the seeds:

a) fruits – 121 fruits were evaluated in 2020 year and 44 fruits in 2021:
   - fruit weight (g), fruit length (mm), fruit width (mm), exocarp thickness on the left side (mm), exocarp thickness on the right side (mm), pericarp thickness on the left side (mm), pericarp thickness on the right side (mm), placenta weight (g), fresh seed weight (g), seed weight after drying (g);

b) seeds – 30 seeds were evaluated from each plant (n = 30);
   - seed length (mm), seed diameter (mm), seed thickness, weight of one thousand seeds (g).

The weights were determined by digital scale (Kern ADB-A01S05, Germany; KERN DS – type D-72336, Kern and Sohn GmbH, Germany), accurate to 0.01 g. Fruits were measured by ruler and seeds were measured by a digital calliper (METRICA 111 – 012, Czech Republic) accurate to 0.02 mm.

Image analysis

a) Shape and colour of fruits.

b) Shape and colour of seeds.

Images were obtained using the stereomicroscope ZEISS SteREO Discovery.V20 (Microlmaging GmbH 37081 Göttingen, Germany), Fuji FinePix S 7000 and Panasonic DMC FZ50 digital cameras.

Statistical analysis

It was evaluated the variability of each character using descriptive statistics. For the characteristics, it was used the basic descriptors of variability: average, minimum measured value, maximum measured value, and the coefficient of variation (%). The degree of variability was determined by the coefficient of variation values. The given parameter is independent of the unit of the evaluated character. Theoretically, they can acquire different values (Stehlíková, 1998). We used analysis of variance (ANOVA) in the program STATISTICA 1.10 to determine the dependence between individual characters.

Results and discussion

Variability of fruit characters

The basic indicators of the variability of quantitative traits are presented in Table 1.

Weight of fruits and weight of placenta (g)

In the experimental year 2020, the weight of the fruit was determined in the range from 521.5 g to 8,698.3 g, and the value of the coefficient of variation indicated a high degree of variability between plants. In 2021, the tested plants reached values of the given trait from 1,031.1 g to 12,744.7 g, and the value of the coefficient of variation indicated a high degree of variability between plants (Table 1).

The weight of the placenta was determined from 20.70 g to 643.6 g, and finally, in 2021, the range of the trait was determined in the range from 89 g to 870 g. The value of the coefficient of variation indicates a high degree of variability between plants in both experimental years. Previously reported fruit mass ranged from 3.5 to 5.0 kg among *Cucurbita landraces* of northern KwaZulu-Natal (Ntuli et al., 2017).

Ruelas Hernándes et al. (2015) studied various *Cucurbita* spp. (*C. pepo*, *C. ficifolia*, *C. argyrosperma*, *C. moschata*). *C. pepo* had the lowest values in weight (0.54–0.8 ±0.22 kg) and width characteristics of fruit, stems and fewer seeds per fruit and with lower values than the other accessions and species. Balkaya et al. (2010) obtained the average weight of fruit *C. maxima* Duch in the interval 3.2–11.8 kg (7.4 ±0.2). Abd El-Hamed (2015) reported three *C. pepo* genotypes and observed fruit weight in the interval 63–242 g with middle and high degree correlation coefficients (14.08–21.4%).

Length and diameter of fruit (mm)

In 2020, the average value of the length of fruit was determined in the range from 85 mm to 246 mm. The value of the coefficient of variation (19.67%) indicates a medium degree of variability. In the experimental year 2021, the plants reached values ranging from 119 mm to 285 mm, and the value of the coefficient of variation indicates a high degree of variability (Table 1).

In 2020, we set the width of the fruit from 114 mm to 3,200 mm and determined a high degree of variability. In the experimental year 2021, the character value was

Table 1 Main statistical indicators of the variability of evaluated fruit traits in the experimental population of *Cucurbita pepo* var. *styriaca*

Traits	Year	min	max	x̄	V%
Weight of fruit (g)	2020	521.50	8698.30	3353.63	55.92
	2021	1031.1	12744.70	4513.32	49.40
Length of fruit (mm)	2020	85.00	246.00	157.07	19.67
	2021	119.00	285.00	181.87	20.11
Width of fruit (mm)	2020	114.00	320.00	204.11	22.43
	2021	135.00	305.00	229.73	18.32
Thickness of exocarp – right side (mm)	2020	0.33	4.56	1.35	51.15
	2021	0.52	2.38	1.30	34.84
Thickness of exocarp – left side (mm)	2020	0.10	4.86	1.16	58.39
	2021	0.58	3.98	1.33	43.47
Thickness of pericarp – right side (mm)	2020	9.77	46.05	26.51	32.77
	2021	13.12	45.85	28.53	29.45
Thickness of pericarp – left side (mm)	2020	9.13	48.41	26.34	32.86
	2021	15.33	57.02	30.42	27.72
Weight of placenta (g)	2020	20.70	643.60	214.45	58.13
	2021	89.00	870.00	340.60	43.11
Weight of fresh seeds (g)	2020	17.30	638.50	134.61	56.57
	2021	67.20	270.00	148.21	38.41
Weight of dried seeds (g)	2020	4.53	187.36	72.02	49.85
	2021	11.57	159.35	67.78	52.31

Note: n – the number of measurements; min, max – minimal and maximal measured values; x̄ – arithmetic mean; V – coefficient of variation (%)

determined in the range from 135 mm to 305 mm with a medium degree of variability.

The ranges for fruit length (27.4–38.6 cm) and fruit diameter (51.8–68.5 cm) were observed among the *Cucurbita landraces* in the previous study (Ntuli et al., 2017). Ruelas Hernándes et al. (2015) reported the width of *C. pepo* in the interval 9.63–9.97 cm. For comparison values from other *Cucurbita* spp. Balkaya et al. (2010) studied *C. maxima* collected from the Black Sea Region of Turkey and determined fruit length and fruit diameter in the interval 26.0–49.8 and 35.1–56.5 cm, respectively. The variation in the fruit length and fruit diameter may be attributed to genetic differences existing among the landraces. Aruah et al. (2010) and Balkaya et al. (2010) reported that *Cucurbita* plants produce fruits of various sizes as detected by the genetic constitution. Abd El-Hamed (2015) observed in *C. pepo* genotypes fruit length in the interval 7–16 cm with small and middle correlation coefficients (7.00–18.24%).

Thickness of exocarp and thickness of pericarp (mm)

The thickness of the exocarp was determined in the first experimental year from 0.10 mm to 4.86 mm and the next year 2021 in the range from 0.52 to 3.98 mm. In general, a high value of the coefficient of variation in this trait was determined in both years.

In 2020, thickness of pericarp was in the range of 9.13 mm to 48.41 mm and 2021 in the range from 13.12 mm to 57.02 mm was recorded for the evaluated trait. High variability prevailed in both evaluated traits.

We recorded a relatively large variability in the shape and colour of fruits of 165 evaluated pumpkins *Cucurbita pepo* var. *styriaca* (Figure 1).

Variability of traits on seeds

At the seed level, a total of four quantitative characters such as the length, width and thickness of the seed and the weight of one thousand seeds were evaluated. Statistical indicators of the variability of the evaluated quantitative traits are presented in Table 2.

Weight of fresh and dried seeds (g)

From the experimental data, the weight of fresh seeds was determined in 2020 in the range from 17.3 g to 638.5 g with a high value of the coefficient of variation (56.57%), which indicates a high degree of variability. In 2021, the values of the given character were reached in the range from 67.2 g to 270 g, and a high value of the coefficient of variation was determined (Table 2).

In the first year, the values of the weight of the seeds after drying were determined in the range from 4.53 g to 187.36 g. The coefficient of variation indicates a very high degree of variability, and thus significant differences between plants. In the second year, the values of the weight of the seeds after drying were determined in the range from 11.57 g to 159.35 g.

The weight ratio of individual parts of the fruit was also evaluated during the years 2020 and 2021 (Figure 2). From the obtained experimental data, the ratio of the basic anatomical parts of the fruit was determined, represented by 89–90% pericarp, 6–8% placenta, and 3% to 4% is the most economically used part of the fruit – seeds.

Table 2 Main statistical indicators of character variability on seeds *Cucurbita pepo* var. *styriaca*

Traits	Year	min	max	$\bar{x}$	V%
Width of seeds (mm)	2020	6.56	14.41	9.23	12.54
	2021	7.24	11.45	9.29	10.42
Length of seeds (mm)	2020	12.91	24.00	17.46	9.76
	2021	14.22	20.53	17.48	9.15
Thickness of seeds (mm)	2020	0.41	3.19	2.22	21.47
	2021	1.43	3.62	2.40	20.03
Weight of thousand seeds (g)	2020	15.41	475.66	191.54	39.32
	2021	21.87	471.44	192.37	42.42

Note: n – the number of measurements; min, max – minimal and maximal measured values; $\bar{x}$ – arithmetic mean; V – coefficient of variation (%)

Figure 1 Variability in the shape and colour of fruits of evaluated pumpkins *Cucurbita pepo* var. *styriaca*

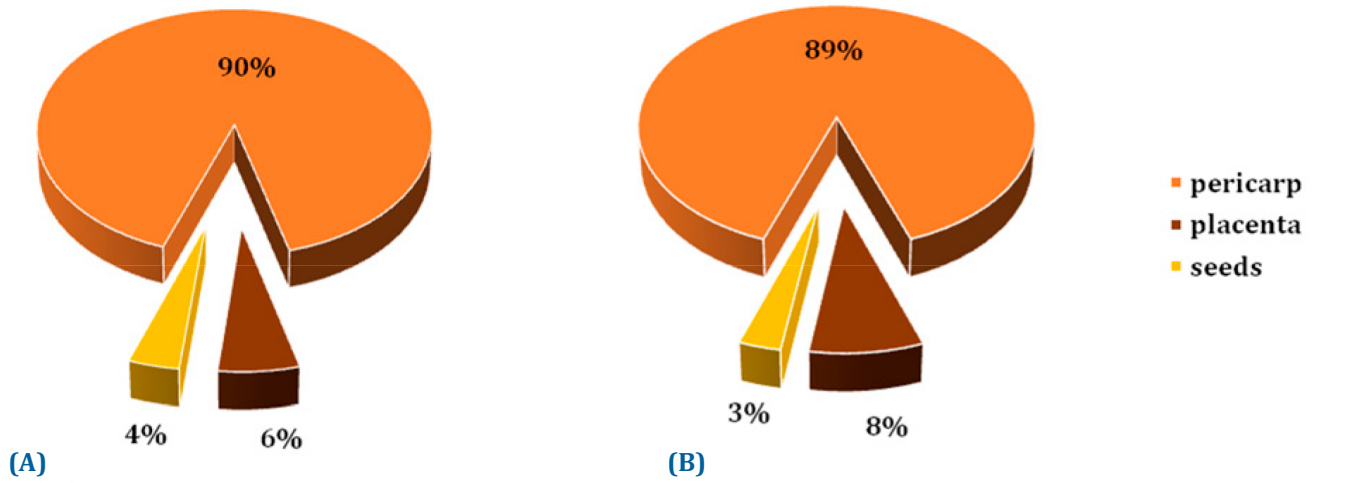

Figure 2 The average weight ratio of the individual basic anatomical parts of the fruits of *Cucurbita pepo* var. *styriaca* from the total average weight of the fresh fruit (A) 2020 and B (2021) (%)

Width and length of seed (mm)

In the first year, the range of seed width was determined from 6.56 to 14.41 mm with a medium degree of variability. In the second experimental year, the value of the trait was determined in the range from 7.24 to 11.45 mm with a medium degree of variability.

Experimental evaluation of another quantitative trait, seed length, in 2020 determined a range from 12.91 to 24.00 mm. And in the next year 2021, the trait was determined in the range from 14.22 to 20.53 mm. In general, a low value of the coefficient of variation was determined in both years (Table 2).

In the previous study by Ruelas Hernándes et al. (2015) length of seed was reported in the interval 12.8–17.4 ±0.59 mm and the width of seed in the interval 9.63–9.97 ±0.76 mm. Abd El-Hamed (2015) determined *C. maxima* seed length and width in the interval 12.68–14.67 mm and 6.66–9.37 mm, respectively with low correlation coefficients (3.15–4.46 and 5.81–7.09%). Paris and Nerson (2003) studied seed samples of 174 accessions of pumpkins, squash as gourds of *Cucurbita pepo* and measured mean seed length ranged from 8.8 to 23.3 mm, mean seed width from 5.0 to 12.5 mm and mean seed thickness from 1.2 to 3.8 mm.

Thickness of seed (mm)

In the first year, the values were determined in the range from 0.41 to 3.19 mm and in the second year in the range from 1.43 to 3.62 mm. High variability prevailed in the evaluated character.

Ruelas Hernándes et al. (2015) observed and measured the thickness of *C. pepo* in the range of 2.43–2.67 ±0.25 mm. Abd El-Hamed (2015) determined *C. maxima* seed thickness ranged from 1.70–3.0 mm with low and middle correlation coefficients (9.80–12.55%).

Weight of one thousand seeds (g)

In the last evaluated character, the weight of one thousand seeds in the range from 15.41 to 475.66 g was recorded in the experimental set in the first year. The coefficient of variation indicates a very high degree of variability and thus significant differences between plants. In the second year, the values of the trait were determined in the range from 21.87 to 471.44 g. A high degree of variability was determined.

Ruelas Hernándes et al. (2015) determined 148–177 ±86 pcs of seeds per fruit and weight of 100 seeds in the range 8.65–13.49 ±6.16 g. The mass of the total fully developed seeds per fruit varied from 48.3 to 70.2 g among *Cucurbita landraces* in northern KwaZulu-Natal and the weight of a 100 seeds of *C. pepo* was in the interval 10.7 ±0.9 – 14.7 ±1.0 g (Ntuli et al., 2017). Abd El-Hamed (2015) determined in *C. maxima* 100 seed weight in the range of 7.89–14.30 g with the law and middle correlation coefficients (8.91–16.46%).

The shape of seeds and colour of seeds are also variable within *Cucurbita pepo* var. *styriaca* plants (Figure 3).

Phenotypic correlation (r) and significance level between fruit morphological traits in *Cucurbita pepo* var. *styriaca* L. evaluated plants are reported in Table 3.

Fruit weight was highly correlated with fruit length (r = 0.966) and fruit width (r = 0.983), and also positively correlated with thickness of exocarp (r = 0.919, r = 0.933) on both sides. Weight of dried

Figure 3 Variability in the shape and colour of seeds of evaluated plants of *Cucurbita pepo* var. *styriaca*

Table 3 Phenotypic correlation and significance level between fruit morphological traits in *Cucurbita pepo* var. *styriaca* L. evaluated plants

Parameters	WF	LF	WF	T TER	TEL	TPR	TPL	WP	WFS	WDS	WS	LS	TS
LF	0.966**	1											
WF	0.983**	0.949**	1										
TER	0.721	0.691*	0.705*	1									
TEL	0.675*	0.634*	0.609*	0.831**	1								
TPR	0.919**	0.947**	0.913**	0.739*	0.593	1							
TPL	0.933**	0.966**	0.925**	0.632*	0.554	0.983**	1						
WP	-0.053	-0.227	-0.101	-0.388	-0.315	-0.179	-0.132	1					
WFS	0.853**	0.797*	0.855**	0.684*	0.489	0.689*	0.676*	-0.075	1				
WDS	0.635*	0.479	0.634*	0.807*	0.720*	0.442	0.368	-0.040	0.754*	1			
WS	0.867**	0.885**	0.879**	0.883**	0.760*	0.932**	0.885**	-0.405	0.667	0.601	1		
LS	0.820**	0.780*	0.907**	0.665*	0.444	0.761*	0.748*	-0.249	0.817*	0.656	0.808*	1	
TS	0.632*	0.530	0.601*	0.789*	0.878**	0.417	0.376	-0.251	0.677*	0.916**	0.619	0.560	1
WST	0.692*	0.606*	0.682*	0.926**	0.867**	0.569	0.489	-0.319	0.729*	0.942**	0.764*	0.677*	0.954**

Notes: WF – weight of fruit; LF – length of fruit; WF – width of fruit; TER – thickness of exocarp right side; TEL – thickness of exocarp left side; TPR – thickness of placenta right side; TPL – thickness of placenta left side; WP – weight of placenta; WFS – weight of fresh seeds; WDS – weight of dried seeds; WS – width of seeds; LS – length of seeds; TS – thickness of seeds; WST – weight of 1000 seeds. **Correlation is significant at p ≤0.01; *correlation is significant at p ≤0.05

seeds was highly correlated with thickness of seeds (r = 0.916). Weight of fresh seeds was non-significantly correlated but with high (r) values with width of seed (r = 0.667), length of seed (r = 0.877) and thickness of seed (r = 0.677). Placenta weight has shown the only negative correlation and non-significant relation with length of fruit (r = -0.227), width of fruit (r = -0.101), thickness of exocarp (r = -0.388, r = -315), thickness of placenta (r = -0.179; r = -0.132) and weight of seeds (r = -0.405).

Conclusions

It is generally known that various types of pumpkins are used in all countries of the world, primarily for the preparation of various simple dishes and various other food products. Of the fruits of pumpkins, pulp and seeds are used the most. Because pumpkins are grown without problems, they are mainly used by poorer groups of the population. That's why pumpkins were given the epithet that they are the "Bread of the Poor". The object of the work was the Styrian pumpkin, which produces valuable and economically important naked seeds. The seeds are the main raw material to produce high-quality oil with high nutritional value. Therefore, *Cucurbita pepo* var. *styriaca* growers usually use only the seeds from the pumpkin fruits, and the pulp and other parts of the fruits remain in the field after harvesting and are then ploughed into the soil. It follows from our results achieved in the experiment that the percentage of the weight of fresh seeds is only 3–4% of the total weight of *Cucurbita pepo* var. *styriaca* fruits. This means that the seed yield is from 300 to 1000 kg from one hectare. While the total harvest of *Cucurbita pepo* var. *styriaca* fruits reaches from 40 to 100 tons per hectare. This means that 95% of the produced biomass remains unused in the field every year. At the same time, results from many literary sources prove that all other parts of unused fruits are very valuable resources for use in the food industry, pharmaceuticals, cosmetics as well as feeding farm animals. The results of our study also unequivocally prove that, although the fruits of the *Cucurbita pepo* var. *styriaca* are not balanced in the evaluated morphological characters and are characterized by a high degree of variability (9.15–58.39%), they are practically usable.

Ethical statements

This article does not contain any studies that would require an ethical statement.

Conflict of interest

None declared.

Funding

The co-author is grateful to Visegrad Fund for the scholarship for the research during which the presented knowledge was obtained.

Acknowledgements

The publication was prepared with the active participation of researchers in the International Network AgroBioNet, as a part of the international program "Agricultural Biodiversity to Improve Nutrition, Health and Quality of Life". The authors are indebted to the departmental colleagues for their support in the development of this manuscript.

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

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Variation of fruits morphometric parameters and iridoid content of *Lonicera caerulea* L. germplasm collection

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Article Details:
Received: 2022-08-25
Accepted: 2022-09-01
Available online: 2022-11-30

DOI: https://doi.org/10.15414/ainhlq.2022.0027

The dark-blue, fleshy berries of *Lonicera caerulea* L. are used as fresh or processed in many products such as juice, smoothie, wine, compote, jam, marmalade, candies, chocolate, cake, or extracts. *L. caerulea* fruits are appreciated in the food industry and medicine mainly due to a high content of biologically active phenolic compounds, especially anthocyanins and vitamin C – in the amount up to 186 mg.100 g^{-1}, and health-promoting properties. Thirty-four genotypes of *Lonicera caerulea* species originated from germplasm collection of the Forest-Steppe of Ukraine in M.M. Gryshko National Botanical Garden of NAS of Ukraine (NBG) (Kyiv, Ukraine) and were characterized by using the morphometric traits and iridoids content. Differences between the genotypes were significant in all observed parameters. The fruits of *L. caerulea* collection varied in evaluated morphometric parameters as follows: fruit weight 0.70–1.54 g, length 15.33–26.52 mm, diameter 7.12–13.10 mm. The shape indexes of fruits varied from 1.42 to 3.65. Our results showed that the content of iridoids ranged from 97 to 314 mg.100 g^{-1} of fresh *L. caerulea* fruits. Interestingly, *L. caerulea* berries taste differs between the varieties, those with bitter or sour-bitter taste are distinguished by the highest content of iridoids (225–314 mg.100 g^{-1}), while berries without bitter taste were characterized by significantly lower contents. Thus, honeysuckle berries should be selected preferentially regarding their bitterness, due to the anti-inflammatory and antioxidative properties of iridoids.

Keywords: honeysuckle, fruits, iridoids, morphometric parameters, genotypes, Ukraine

Introduction

Fruits (berries) are extremely important and valuable components of the human diet, to maintain good health and well-being. The main goal for the food industry and food and nutrition research is to deliver to the marketplace new, safe, highly accepted by consumers and convenient food products with good nutritional and health-promoting properties. Recently, non-traditional and underutilized edible medicinal plants (Vergun et al., 2019; Buyun et al., 2021; Stefanowski

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et al., 2021), especially their fruits rich in bioactive components gained much attention (Klymenko et al., 2017; Latocha, 2017; Lachowicz et al., 2019; Zhurba et al., 2021). Among them should be emphasized fruits of honeysuckle (*Lonicera caerulea* L.).

Nowadays *Lonicera caerulea* is cultivated as an edible plant (Skvortsov, 1986; Grygorieva et al., 2021), belonging to polymorphic species and is regarded by some authors as a complex of microspecies or geographical races, including *L. altaica* Pall., *L. pallasii* Ledeb., *L.* × *subarctica* Pojark., *L. edulis* Turcz. ex Freyn, *L. stenantha* Pojark., *L. buschiorum* Pojark., *L. baltica* Pojark., *L. turczaninowii* Pojark. and *L. kamtschatica* (Sevast.) Pojark. It should be highlighted that significant progress has been made in the industrial cultivation of *L. caerulea* and its processing in China (Huo et al., 2005; Zhao et al., 2015). Also, numerous studies on *L. caerulea* were conducted in European countries, such as the Czech Republic, Estonia, Poland, Slovakia, Romania, and Lithuania (Smolik et al., 2010; Jurikova et al., 2012; Bieniek et al., 2021; Grygorieva et al., 2021). The fruits of *L. caerulea* are used fresh or processed in many products such as jam, marmalade, jelly, compote, cake, juice, sauce, extracts, liqueur, smoothie, and wine (Liu et al., 2010; Klymenko et al., 2017; Senica et al., 2019).

The *L. caerulea* is highly appreciated for ultra-early fruit ripening, as well a high content of biologically active compounds (Khattab et al., 215; Peng et al., 2016; Kucharska et al., 2017; Bieniek et al., 2021; Cheng et al., 2022; Piekarska et al., 2022) with strong antioxidant (Bąkowska-Barczak et al., 2007; Gruia et al., 2008; Celli et al., 2014; Gao et al., 2016; Hsu et al., 2016), anti-inflammatory (Xu et al., 2007; Hsu et al., 2016; An et al., 2020), immunomodulating (Svarcova et al., 2007), antiviral (Svarcova et al., 2007), antifungal (Palikova et al., 2008), antiallergic (Svarcova et al., 2007), antibacterial (Celli et al., 2014), and immunotropic (Piekarska et al., 2022) properties.

Iridoids are natural secondary metabolites of plants, belonging to the chemical group of heterocyclic monoterpenoids. Iridoids are widespread mainly in the green parts of plants, therefore their presence in edible fruits is scarce. There are only a few reports devoted to the content of iridoids in the genus *Lonicera*, thus the content of iridoids of *Lonicera caerulea* fruits remains to be well-studied. Thus, this study aimed to determine the iridoid content and morphometric parameters of *Lonicera caerulea* of 34 genotypes originated from germplasm collection of the Forest-Steppe of Ukraine in M.M. Gryshko National Botanical Garden of NAS of Ukraine (Kyiv, Ukraine), simultaneously pointing the best genotypes which can be successfully applied as a novel plant source of functional foods.

Material and methodology

Collection of plant material

Berries of *Lonicera caerulea* (Figure 1) were harvested in the full maturity stage and collected in 2022 from the 13–18-year-old plants growing in the Forest-Steppe of Ukraine of Department of Fruit Plants Acclimatization in M.M. Gryshko National Botanical Garden of NAS of Ukraine (NBG) (Kyiv, Ukraine). Thirty-four genotypes (LC-01–LC-34) of *Lonicera caerulea* species were evaluated.

Morphometric characteristics

Pomological characteristics of ripened fruits were conducted with four replications on a total of 120 fruits per genotype. In our experiments, only one plant was used per genotype. In total 4080 fully ripened fruits of *L. caerulea* were investigated. Morphometric parameters were evaluated as follows: fruit weight (g), fruit length (mm), and fruit diameter (mm). The length and diameter of the fruits were measured using a digital calliper Kronos KM-DSM-200 (0–200/0.01; ±0.02 MM). The fresh fruit weights were determined using an analytical balance (Kern ADB-A01S05, Germany).

Chemicals

All chemicals and reagents were of analytical grade and were purchased from Merck (Darmstadt, Germany) and HIMLABORREACTIVE (Ukraine).

Iridoids content

The content of iridoids was determined in the fresh fruit extracts of 34 *L. caerulea* genotypes. Identification of iridoids was performed by the interaction of iridoid compounds with hydroxylamine and the formation of the oxime. The resulting oxime reacts by complexation with trivalent iron cations. The maximum absorption of the complex was measured at 512 nm (photoelectrocolorimeter Zalimp KF 77; Poland), with harpagid as the control.

Extraction was carried out with chloroform: ethanol mixture (5:1), and after the removal of the solvent, the residue was extracted with water. The change in the solvent made it possible to avoid the influence of concomitant substances on the results of the

Figure 1 Variability in the shape of *Lonicera caerulea* L. fruits

hydroxame reaction (Ivanova et al., 2010). Obtained data was calculated using the following equation:

$$C_{irid} = \frac{D \times K}{56 \times m(100 - W)}$$

where: D – the optical density of the solution; V – the total amount of the extract and the average sample (ml); m – linkage, the average of the sample (g); K – conversion factor; 56 – the specific absorption index of the products of the reaction of harpagide with hydroxylamine and iron (III) chloride; W – raw material humidity

The accuracy of the method was in the range of 2.5–4.8%. The results were expressed as mg.100 g^{-1} of fresh weight (FW) in terms of anthocyanidin.

Statistical analysis

All analyses were performed in triplicate. The results were presented as mean ± standard deviation (SD). Hierarchical cluster analyses of similarity between genotypes were computed by the Bray-Curtis similarity index and were performed using PAST 2.17 software (Norway, 2001).

Results and discussion

Fruit pomological properties

It should be pointed out that there are more than 40 genotypes of seed origin from the European part of Russia, the Kuril Islands, and Canada in the germplasm collection of the M.M. Gryshko National Botanical Garden of NAS of Ukraine (Kyiv, Ukraine). The fruits of the *Lonicera caerulea* collection varied in weight,

Table 1 The variability of morphometric parameters of all *Lonicera caerulea* L. genotypes

Characteristics	n	min	max	x̄	V%
Fruit weight, g	4080	0.70	1.54	1.04	19.74
Fruit length, mm	4080	15.33	26.52	20.66	17.10
Fruit diameter, mm	4080	7.12	13.10	9.93	12.37
Shape index	4080	1.42	3.65	2.11	22.49

Note: n – the number of measurements; min, max – minimal and maximal measured values; x̄ – arithmetic mean; V – coefficient of variation (%)

shape, size, the color of fruits, and also degree of the wax coating were noted (Figure 1).

The biometric values for the weight, length, diameter, and shape index of fruits of 34 *Lonicera caerulea* genotypes are shown in Table 1. Fruit weight, which is economically the most important characteristic of fruits, ranged from 0.70 (LC-18) up to 1.54 g (LC-13). Morphological variation of fruit length varied between 15.33 mm for genotype LC-24 and 26.52 mm for genotype LC-06 (Table 1, Figure 2, 3, 4). The values of diameter varied within the interval from 7.12 mm (LC-27) to 13.10 mm (LC-16).

Fruit weight and size are primarily phenotypic features and reflect the impact of environmental growth conditions, while the fruit shape index is a genetically fixed feature. It is on this basis that some subspecies of *L. caerulea* were previously identified as distinct species (Grygorieva et al., 2021).

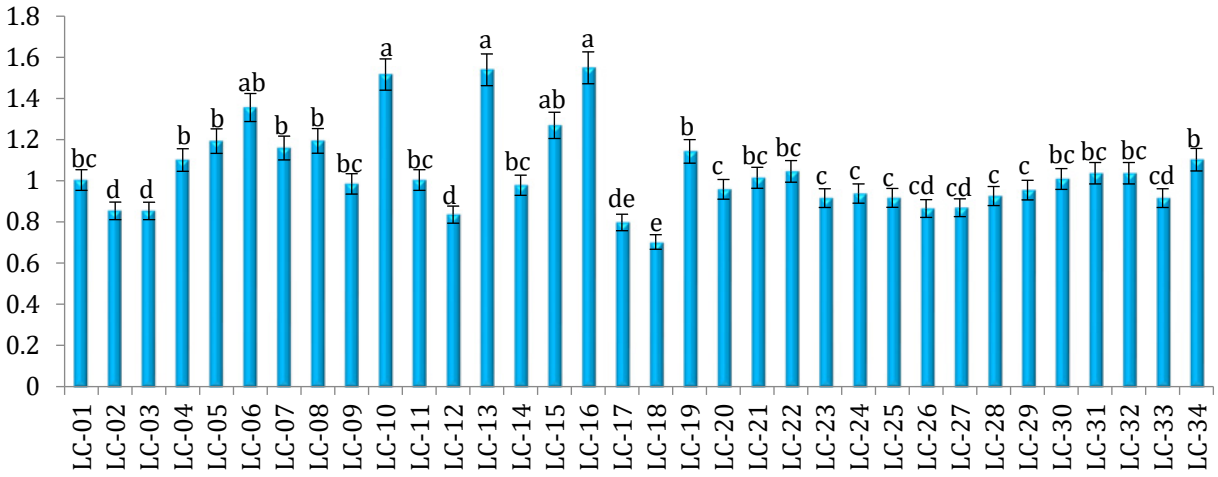

Figure 2 *Lonicera caerulea* L. fruits weight of 34 genotypes (means values ± SD); different superscripts in each column indicate the significant differences in the mean at p <0.05

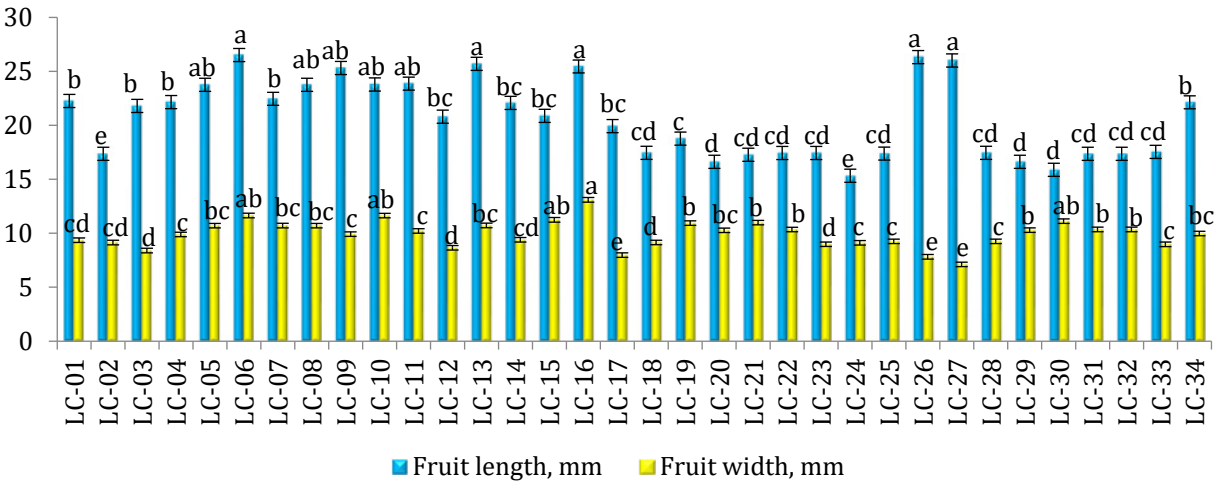

Figure 3 *Lonicera caerulea* L. fruits length and width of 34 genotypes (means values ± SD); different superscripts in each column indicate the significant differences in the mean at p <0.05

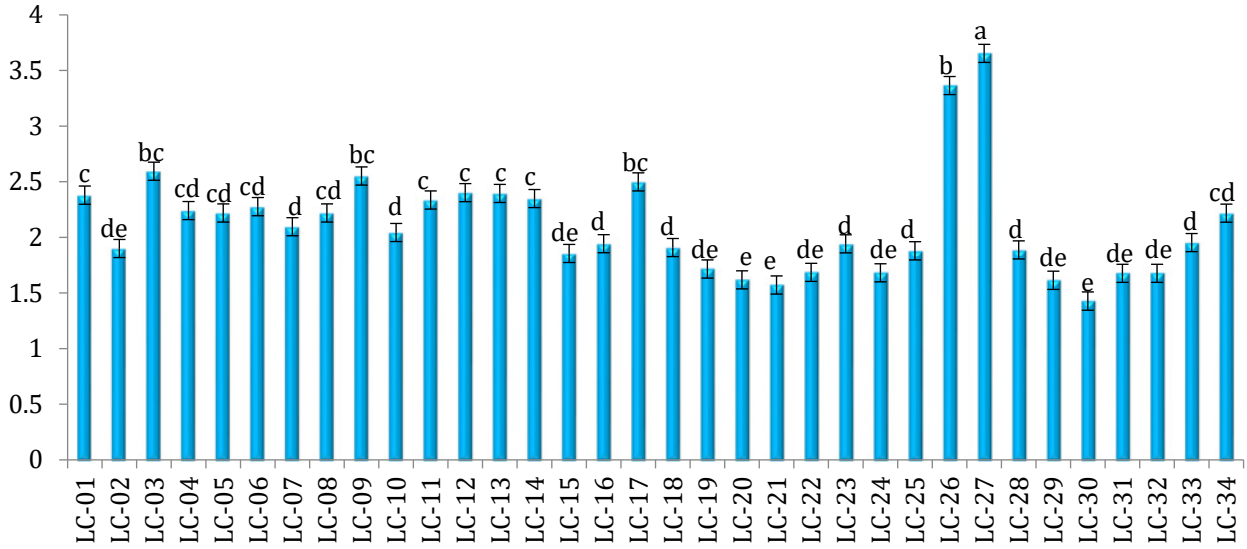

Figure 4 Comparison of shape index of *Lonicera caerulea* fruits 34 genotypes; different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

The shape index average values of *Lonicera caerulea* fruits ranged from 1.42 (LC-60) to 3.65 (LC-57) (Figure 4). These results are in agreement with the study of Grygorieva et al. (2021) who determined the shape index of *L. caerulea* fruits (1.51–3.52).

Differences in the weight of *Lonicera caerulea* fruits were also previously reported by Plekhanova (2000), Thompson and Barney (2007), Fu et al. (2011), Gawronski et al. (2014), MacKenzie et al. (2018), and Holubec et al. (2019) who found the weight ranged from 0.21 to 2.70 g of different plant genotypes. A study by Fu et al. (2011) showed that the length of fruits was in the range of 11.16–19.43 mm, and Senica et al. (2018) detected values in the interval of 18.10 to 26.32 mm. Investigations by Holubec et al. (2019) established the range of fruit length of varieties from 15.50 to 20.40 mm. According to the previously studied collection of 26 *L. caerulea* genotypes (Grygorieva et al., 2021), the morphometric parameters were as follows: fruit weight from 0.73 to 1.60 g, fruit length from 16.42 to 27.29 mm, fruit diameter from 7.77 to 12.34 mm. The presented results are in accordance with the previously published studies and strongly support the statement that between *L. caerulea* genotypes exists high variability in morphometric parameters.

The analysis of the coefficient of variation showed a significant level of variability of morphological parameters between studied *L. caerulea* samples. The variation coefficients (%) ranged between 14.35 (LC-55) and 31.04 (LC-03) for fruit weight, between 7.31 mm (LC-50) and 16.35 (LC-04) for fruit length, between 6.99 (LC-60) and 18.54 (LC-04) for fruit diameter, and between 4.21 (LC-56) and 17.88 (LC-01) for the shape index. Data clearly showed that the most variable is fruit weight.

Iridoids content in *Lonicera caerulea* fruits

Iridoids are known plant-derived compounds mainly for the variety of their health-promoting properties. Pharmacological studies devoted to the isolation and application of iridoids from different plants proved their valuable effect on human health, namely antioxidative, anti-inflammatory, anti-cancer, anti-atherogenic, antidiabetic, neuroprotective, antimicrobial, diuretic, sedative, hepatoprotective, hypolipidemic, neuroprotective, and purgative activities (Tundis et al., 2008; Dinda et al., 2011; Viljoen et al., 2012).

Regarding the fact, that iridoids are mainly found in the green parts of plants, such as leaves and young stems, but only occasionally can be present in fruits and shoots (Dinda et al., 2007; Villasenor, 2007). However, there are some exceptions to this rule, e.g., fruits *Vaccinium macrocarpon* Aiton (Turner et al., 2007), *Vaccinium myrtillus* L. (Juadjur and Winterhalter, 2012), *Cornus mas* L. (Kucharska et al., 2015), *Cornus officinalis* Torr. ex Dur. (Klymenko et al., 2021).

Among the species of the genus *Lonicera*, iridoid compounds were identified mainly in the leaves of *L. caerulea* (Machida et al., 1995a, b) and different morphological parts of *L. japonica* (like e.g. flowers, buds, stem, leaves, and caulis) (Qi et al., 2009; Guo et al., 2014; Ye et al., 2014; Zhang et al., 2015). It should be highlighted that, there are published only a few reports devoted to the content of iridoids in the fruits

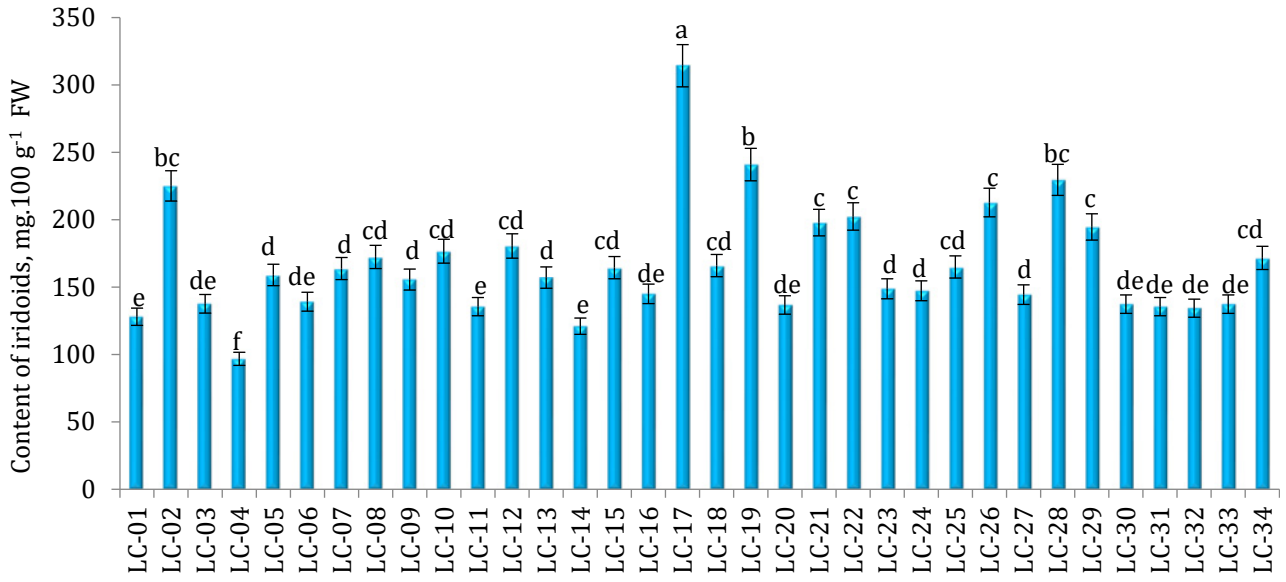

Figure 5 Content of iridoids in fruits of various genotypes of *Lonicera caerulea* L; different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

of the genus *Lonicera* (Whitehead and Bowers, 2013; Kucharska and Fecka, 2016; Kucharska et al., 2017).

In our study, the iridoid content in all studied *Lonicera caerulea* genotypes ranged from 96.78 (LC-04) up to 314.32 mg.100 g^{-1} FW (LC-17) (Figure 5). Interestingly, the *L. caerulea* berries taste differs between the varieties (data not shown). Those with bitter or sour-bitter taste are distinguished by the highest content of iridoids (225–314 mg.100 g^{-1} FW), while berries without bitter taste were characterized by significantly lower contents. It should be noted that the exceptionally high content of iridoids was observed in one sample – LC-17 genotype (314 mg.100 g^{-1} FW). Sensory evaluation of these fruits revealed that LC-17 genotype fruits are characterized by extremely bitter taste (data not shown). The LC-19 genotype also represents a fairly high content of iridoids – 241 mg.100 g^{-1} FW. According to the sensory analyses, the fruits of this genotype were characterized as sour-bitter (data not shown). Genotypes LC-02 and LC-28 have an iridoid content of 225 and 230 mg.100 g^{-1}, respectively and the sensory estimation proved that these fruits were slightly bitter (data not shown). Thus, honeysuckle berries should be selected preferentially regarding their bitterness, due to the anti-inflammatory and antioxidative properties of iridoids.

The study of Kucharska et al. (2017) was devoted to the identification of the iridoids profile of 30 different honeysuckle berry cultivars and genotypes with an application of UPLC-ESI-qTOF-MS/MS combined with HPLC-PDA. It should be highlighted that some compounds, like 8-epi-Loganic acid, pentosyl-loganic acid, taxifolin 7-O-dihexoside, and taxifolin 7-O-hexoside were detected in honeysuckle berries for the first time. The Kuvshinovidnaya cultivar was distinguished by the highest content of iridoids (372 mg.100 g^{-1} FW). In the iridoid profile, loganic acid was assayed as dominated compound (even up to 73% of the total amount of quantified iridoids) in honeysuckle berries (Kucharska, et al., 2017). In the experiment of Perova et al. (2019) who analyzed 15 frozen fruit samples of *Lonicera edulis* collected in Tambov, Voronezh, Moscow regions, and Karelia, total iridoids content ranged from 78 to 342 mg.100 g^{-1}. Accordingly to literature data and the results presented in this study, we can conclude that *Lonicera caerulea* berries proved to be rich in iridoids and the content of iridoids markedly differed between *Lonicera caerulea* genotypes.

The amounts of iridoids in *L. caerulea* were generally much lower than in cornelian cherry (*Cornus mas* L.) ripe fruits, in which the content of total iridoids ranged from 86.91 to 493.69 mg.100 g^{-1} FW (Kucharska et al., 2015). Extremely rich in iridoids turned out to be fruits of *Cornus officinalis* with the content of four main iridoids in the range of 1002–3819 mg.100 g^{-1} (Liu et al., 2012). Moreover, the total iridoids content in many other fruits covered a wide range, i.e. from 89.09 (*C. mas* cv. Ekzotychnyi) to 1441.22 mg.100 g^{-1} FW (*C. officinalis*, Co-01). The average iridoids contents in the analyzed *C. mas*, *C. officinalis*, and *C. mas* × *C. officinalis* fruits were 190.11, 1117.01, and 293.47 mg.100 g^{-1} FW, respectively (Klymenko et al., 2021). Whitehead and Bowers (2013) determined

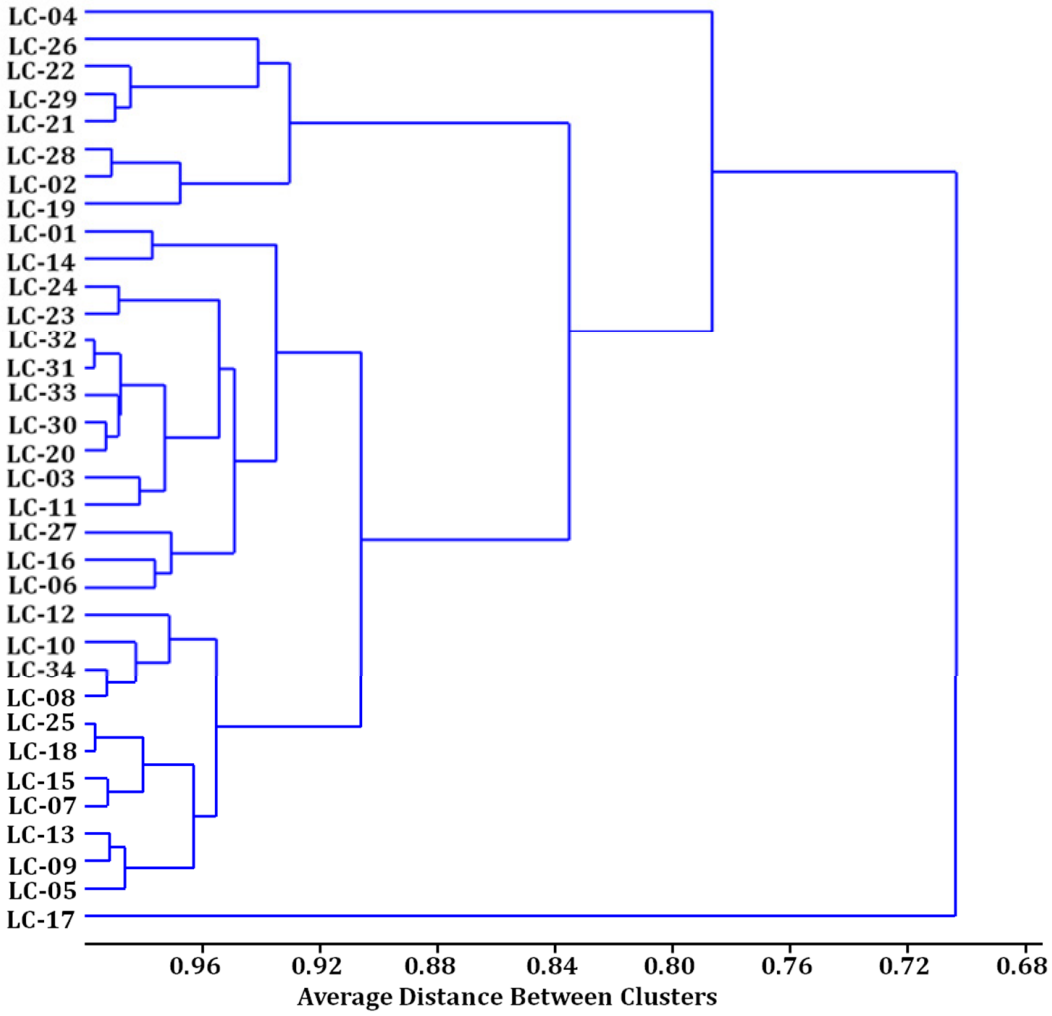

Figure 6 The cluster dendrogram analyzed on the morphometric parameters and iridoid content of 34 genotypes of *Lonicera caerulea* fruits

sixcompounds from this group in non-edible fruits from the species *Lonicera morrowii* A. Gray, *Lonicera tatarica* L., and their hybrid *Lonicera* × bella Zabel.

Based on the data obtained in our study we provided the determination of relatedness by the method of discriminant analysis (Figure 6). A comparison clearly shows the different genotypes and grouping and the significant differences between them.

Conclusions

This study demonstrates that *Lonicera caerulea* L. fruits may be regarded as a valuable plant source of biologically active compounds – iridoids (from 97 (LC-04) to 314 mg.100 g^{-1} FW (LC-17). However, it should be highlighted that studied *Lonicera caerulea* genotypes (34) available from M.M. Gryshko National Botanical Garden of NAS of Ukraine (Kyiv, Ukraine) differed significantly in all morphological parameters and iridoid contents. Interestingly, *L. caerulea* berries taste differs between the varieties, those with bitter or sour-bitter taste are distinguished by the highest content of iridoids (225–314 mg.100 g^{-1}), while berries without bitter taste were characterized by significantly lower contents. Thus, honeysuckle berries should be selected preferentially regarding their bitterness, due to the anti-inflammatory and antioxidative properties of iridoids. Moreover, the presented germplasm collection has significant genotypic potential for further selection for adaptability and improvement of fruit quality.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

Funding

This work was supported by the grant Visegrad Fund and SAIA (Slovakian National Scholarship Program) for the scholarship for the research during which the presented knowledge was obtained.

Acknowledgements

The publication was prepared with the active participation of researchers in the International Network AgroBio*Net*.

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Review

OPEN ACCESS

Water in biological and food systems

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Article Details:
Received: 2022-11-13
Accepted: 2022-11-25
Available online: 2022-11-30

DOI: https://doi.org/10.15414/ainhlq.2022.0028

This article presents data on the physicochemical characteristics of water in biological and food systems such as redox potential, water activity, water potential; and their influence on the processes occurring in plant materials and processed products. According to the laws of thermodynamics, water in biological and food systems is in a stationary state. Entropy changes in biological and food systems do not occur during reversible phase transitions of water. In all studied samples (E >0), despite the rather high content of antioxidants (polyphenols and L-ascorbic acid) in plant raw materials. The only exception was sweet pepper E= -10–+50 mV. The relatively high content of L-ascorbic acid, up to 102 mg.100 g^{-1}, led to a decrease in the redox potential and a shift in redox reactions towards an increase in the reduction potential of the system water – L-ascorbic acid (E ≤0). Bound water has anomalous properties, for example: its density increases, loses the properties of a solvent, fluidity is lost, the boiling point is above 100 °C. In such an environment, biochemical, chemical and biological processes proceed at a low rate, and at a_w ≤0.2 these processes stop completely. Alkaline mineral water belongs to the hydrocarbon group of water obtained from natural sources. The acidity of water exceeds pH 7. Because of the predominance of bicarbonate ions (HCO_3^-), sodium, potassium, magnesium and other minerals in it, the water is conditionally called alkaline, and its beneficial properties are used to treat several diseases. Water has unique properties associated to a certain extent with the polarity of its molecules and their ability to form hydrogen bonds with each other. Therefore, the electrical polarity of liquid water molecules has the property of forming many hydrogen bonds with a total strength between the molecules.

Keywords: water potential, redox potential, water activity, water structure, entropy

Introduction

Water is the main component of most foods. It has a dominant influence on many quality indicators, especially those related to the texture and shelf life of food products. The quality of potable water, including the quality and healing properties of mineral waters, is also of exceptionally great importance. During the processing of vegetable raw materials, various food products containing biologically active substances necessary for the human body can be obtained. In this regard, it is appropriate to consider the role of water in the physicochemical processes occurring in plant materials and food products. Considering that plant raw materials and the assortment of food products are quite diverse, it seems appropriate to consider them as biological and food systems, which are characterized by generalized indicators and their elements, in particular, the state and behaviour of water.

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A set of interrelated and mutually influencing elements with joint action, which are the object of thermodynamic research, is called a thermodynamic system. In particular, fruits, berries, vegetables are complex biological systems of different levels of the organization. Several food products are derivatives of biological systems and our food systems. Previous studies have established that the physical and biological state of fruits, berries, vegetables, obey the fundamental laws of thermodynamics, in particular, they are characterized by a state function – entropy (Opritov, 1999; Masimov, 2018; Etkin, 2019).

The only possible form of functional activity of any system, including biological and food systems, is the life cycle. Biological systems are open non-equilibrium thermodynamic systems, consisting of a set of hierarchically interconnected components, which includes the dynamics of development from inception to the cessation of existence (Ondar, 2011). The life cycle has a beginning and an end; and includes the inception of the system, its formation and continuous development, maturity, reproduction, ageing and cessation of activity. During the life cycle, biochemical reactions constantly and purposefully proceed, aimed at the formation in the structure of fruits and vegetables the embryos, grains, seeds, which contain the genetic codes for the reproduction of the former system. The further existence of the biological system continues due to new generations. A group of systems with approximately the same life cycle in biology is called a species or population (Etkin, 2021; Henry, 2021; Helson and Cox, 2022).

Food products obtained by technological processing of plant raw materials are thermodynamic multicomponent closed systems with a specific life cycle of reproduction, which includes all stages from the idea of obtaining a product, the formation of a product structure, its production technology, transportation, storage time, ageing and its complete degradation after a storage period. The quality, nutritional and biological value of a food product depends on the interaction of all its components (ISO/IEC 15288, 2008; ISO/IEC 42010, 2011).

A special role in the functioning of these systems plays water. Due to its specific chemical structure and its special properties, water has a dominant influence on the vital functions of biological systems and the stability of food systems (Tatarov and Rusu, 2002; Masimov, 2018; Zemskov, 2018).

The mass fraction of water in food products ranges from 2.0 to 97.0%. Typically, water in biological and food systems is in a bound state. The types and state of bound water have different effects on the stability of food systems and the manner in their change over time. Numerous papers published recently are devoted to the study of water and its properties, as well as the influence of water structure and its physical characteristics on the functions of biological systems (Etkin, 2003; Tijskens, 2004; Korotkov, 2019).

From the point of view of theoretical aspects and applied value, it is of interest to consider the physicochemical state of water in certain types of plant raw materials and their processed products. This work presents some considerations about the physical characteristics of water such as redox potential, water activity, as well as indicators of water state in biological and food systems.

Redox potential of water

Water is involved in redox reactions in both food and biological systems. In a living organism during the biological oxidation of food components such as proteins, fats and carbohydrates, through enzymatic redox reactions, chemical bonds are split with the release and accumulation of released energy in molecules of adenosine triphosphate (ATP) (Nelson and Cox, 2022). In food systems, water is the medium in which redox reactions occur, mainly, in the direction of oxidation of phenolic substances, water-soluble vitamins, pigments, etc. (Martinovich and Cherenkevich, 2008; Tatarov, 2017).

Water is a weak electrolyte and is an equilibrium system consisting of water molecules, H^+, HO^- ions. The total reaction of the equilibrium state of system elements is:

$$O_2 + 4H^+ + 4e \rightleftarrows 2\ H_2O \qquad (1)$$

In accordance with equation (1), water molecules are in a reduced state, molecular oxygen and H^+ ions are in an oxidized state. The equilibrium state of water is achieved as a result of the exchange of electrons between the reduced (Red) and oxidized (Ox) components:

$$Ox + ne \rightleftarrows Red$$

Redox potential represents the chemical activity of water components in the oxidation-reduction reaction. This dependence is represented by the Nernst equation (Rubin, 1987; Sandulachi and Tatarov, 2012).

$$E = E^0 + \frac{RT}{nF} Ln \frac{\left[H^+\right]^4 \left[O_2\right]}{\left[H_2O\right]^2} \quad (2)$$

where: E – redox potential of water, mV; E° – standard value of water redox potential, mV. (E° = 815 mV); R – universal gas constant (R = 8.314 $J.K^{-1}.mol^{-1}$); T – temperature, °K; $[O_2]$ – dissolved oxygen concentration, $mol.dm^{-3}$; $[H^+]$ – hydrogen ion concentration, $mol.dm^{-3}$; $[H_2O]$ – water concentration, $mol.dm^{-3}$; n – number of electrons; F – Faraday constant (F = 96.485 $C.mol^{-1}$)

In accordance with the Nernst equation (2), the redox potential is a generalized index of the state of liquid water, depending on the ratio (Ox/Red):

$$E = E^0 + \frac{RT}{nF} lg \frac{\left[Ox\right]}{\left[Red\right]} \quad (3)$$

As a result of experimental data analysis, the redox potential of distilled water corresponds to the following equation:

$$E = E° - 59.16\ pH + 14.79\ lg\ [O_2] \quad (4)$$

These data indicate the fact that the state of water is shifted to the region of the oxidized state E >0. Thus, pure water is an environment that has oxidizing properties and promotes the oxidation of organic substances and reduces the activity of antioxidants. The experimental data presented in Table 1 indicate different values of the redox potential of water in the studied fruits, vegetables and berries (Tatarov and Rusu, 2002).

A common indicator of the studied fruits, vegetables and berries is the oxidized state. In all studied samples (E >0), despite the rather high content of antioxidants (polyphenols and L-ascorbic acid) in plant raw materials. The only exception was sweet pepper E = -10–+50 mV. The relatively high content of L-ascorbic acid, up to 102 $mg.100\ g^{-1}$, led to a decrease in the redox potential and a shift in redox reactions towards an increase in the reduction potential of the system water – L-ascorbic acid (E ≤0).

The redox potential of the system water – L-ascorbic acid depends on the standard value of the redox potential of water, as well as on the standard value of the redox potential of L-ascorbic acid, the pH of the medium, the concentration of dissolved oxygen. In general, the redox potential of the system water – L-ascorbic acid is determined by the following equation:

$$E°° = E°_в - E°_{AA} - 59.16\ pH + 44.35\ lg\ [O_2] + 414.40 \quad (5)$$

where: E°° – redox potential of the system water – L-ascorbic acid, mV; $E°_в$ – standard value of water redox potential, $E°_в$ = 815 mV; $E°_{AA}$ – standard value of L-ascorbic acid redox potential, mV

The standard value of L-ascorbic acid redox potential depends on the pH value of medium:

$$E°_{AA} = 355 - 25\ pH$$

In the pH range of 3.0 ... 7.0, standard values of redox potential for L-ascorbic acid are:

$$E°_{AA} = 180 \ldots 280\ mV$$

The standard values of redox potential of the system water – L-ascorbic acid in the pH range of 3.0 ... 7.0 are:

$$E°° = 539 \ldots 553\ mV$$

Table 1 Redox potential of fruits, vegetables and berries (Tatarov and Rusu, 2002)

Species	Redox potential, mV	Water content, %	pH	Antioxidant content, $mg.100\ g^{-1}$	
				L-ascorbic acid	polyphenols
Aronia	220–225	81–82	3.9	42–45	1100
Sea buckthorn	115–136	88–89	2.9	18–21	320
Sweet pepper	-10–+50	92–94	5.6	75–102	–
Tomatoes	190–230	94–95	4.3	5.8–7.1	–
Quince	180–200	96–98	4.2	16.0–17.6	250
Plum	320–340	89–90	3.7	2.3–3.5	200–400
Peach	330–340	90–92	4.1	7.2–8.6	160
Strawberry	128–145	87–93	3.3	32–46	280–360
Raspberry	214–257	84–89	3.6	30–40	235–330

The standard values of redox potential of the system water – L-ascorbic acid ($E^{\circ\circ}$) are higher than the standard values of L-ascorbic acid redox potential (E°_{AA}).

$$E^{\circ\circ} > E^{\circ}_{AA}$$

This ratio once again testifies to the physicochemical properties of water in biological and food systems, in particular, it leads to a decrease in the reduction potential of L-ascorbic acid and other antioxidants. However, it should be noted the special function of water in the process of obtaining energy by living organisms through the biological oxidation of proteins, carbohydrates, and fats and in metabolic processes in biological systems.

Water activity

In biological and food systems, water is one of the key components. Along with the redox potential, the physicochemical properties of water are also determined by an indicator called water activity (a_w) (Smirnov and Trongsawad, 2006; Shishkov, 2009; Masimov, 2018).

Water activity is a thermodynamic indicator of the interaction of water with chemical components in biological and food systems. The activity of biological systems and, in particular, food systems, is determined not by the amount of water in them, but by its thermodynamic state. Water molecules have kinetic energy, which depends on their internal state. Based on the second law of thermodynamics, the internal energy of water is conditionally divided into free (F) and bound (ST) energy. Free energy is characterized by the chemical potential of water (Rubin, 1987).

The chemical potential characterizes the amount of free energy in $J.mol^{-1}$, which 1.0 mol of water has. The chemical potential of water is determined by the equation:

$$\mu = \mu_0 + RT \ln a \qquad (6)$$

where: μ – chemical potential of water; μ_0 – chemical potential of water in standard state (25 °C, 1 atm); R – gas constant; T – absolute temperature; a – thermodynamic activity of water

The decrease in the free energy of water (ΔF) is accompanied by a change in the chemical potential from the value of μ_0 to the value of μ, as a result of energy consumption for desorption processes:

$$\Delta F = \mu - \mu_0 = - RT \ln a = -RT \ln P_m/P_u = -RT \ln \varphi \qquad (7)$$

where: ΔF – value of decreasing in the free energy of water; P_m – partial pressure of moisture vapour on the system surface in equilibrium; P_u – partial pressure of moisture vapour in the environment; φ – relative humidity, %

According to equation (7), the decrease in the free energy of water depends on the value of relative humidity (φ) on the system surface and in the environment. According to the modern concept, water activity is determined by the ratio of the water vapour pressure on the surface of the system (product) to the moisture vapour pressure in the environment at the same temperature in an equilibrium state:

$$a_w = \frac{P_m}{P_u} \qquad (8)$$

The numerical values of a_w vary within 0... 1.0 or 0... 100%. In cases where the partial pressure of moisture vapour on the surface approaches zero ($P_m \rightarrow 0$), water is in a bound state with the components located on the product surface. In this case, water does not evaporate $a_w \rightarrow 0$.

Water in the liquid state, at $a_w \rightarrow 1$, is the medium in which biochemical, chemical and biological processes actively proceed. The decrease in water activity in food and biological systems is a consequence of the transition of water molecules to a bound state. Bound water is a structured medium in the form of ordered layers of molecules, physically and chemically related to macromolecules of carbohydrates, proteins and other chemicals. The more macromolecules with polar groups on the surface, the higher the degree of hydration.

Bound water has anomalous properties, for example: its density increases, loses the properties of a solvent, fluidity is lost, the boiling point is above 100 °C, etc. In such an environment, biochemical, chemical and biological processes proceed at a low rate, and at $a_w \leq 0.2$ these processes stop completely.

At present, the indicator of water activity is widely used in the technology of production and quality control of food products. Depending on the value of water activity a_w, categories of food products with different stability and shelf life are established (Tatarov, 2017).

The shelf life of products at a temperature of 20 °C, depending on the amount of water activity is:

- from 2 to 5 years or more possess dehydrated (dried) food products with a_w = 0.3... 0.6;

- up to 1 year possess foods with intermediate moisture content and a_w = 0.6... 0.85.

The above products are microbiologically, chemically, physically stable and retain organoleptic properties.

The shelf life of food products with a water content of more than 65%, a_w = 0.97... 1.0 (fruits, berries, milk, meat, etc.) at t = 20 °C ranges from several hours to 2–3 days.

As an example, the stability of the reducing antioxidant activity of vitamin C (L – ascorbic acid) in solutions with different water activities is shown (Figure 1). The system water - vitamin C, with water activity, a_w = 1.0, leads to a complete loss of vitamin C activity within 40 days of storage at t = 20 °C.

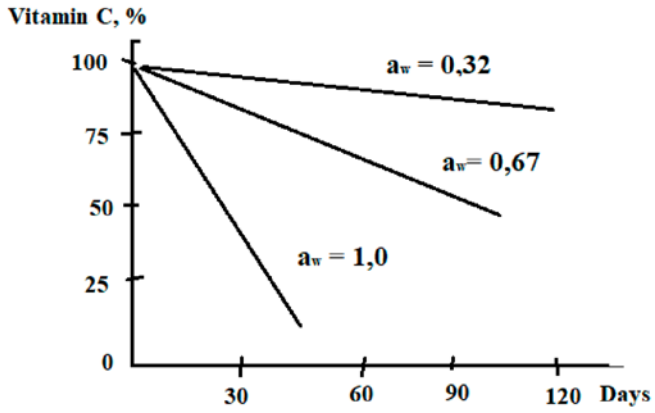

Figure 1 Change in the reducing activity of vitamin C depending on the activity of water at t = 20 °C

Given the importance and great influence of water activity on the stability and shelf life of food products, this indicator (a_w) is included in the system of international standards (ISO 9000, 2015).

Some biological properties of water

In biological systems, liquid water retains the basic physical and chemical properties: structure, boiling and freezing points, density, phase transformations, heat capacity, etc. One of the main elements of biological systems of plant origin is a plant cell. The physiological activity of a cell is determined not by the amount of water, but by its thermodynamic state (Shishkov, 2009). Plants grown at the same humidity and containing almost the same amount of water in the tissues, but at different levels of mineral nutrition, have a different thermodynamic (energy) state. The thermodynamic state of water in biological systems is judged by the magnitude of its chemical potential, water potential, osmotic potential, and pressure potential (Etkin, 2003).

Water potential is a measure of the difference between the free energy of water inside the cell and outside the cell, at the same temperature and pressure. It should also be noted that the difference between the chemical potential of water in a cell (μ) and the chemical potential of pure water (μ_0), the difference $\mu - \mu_0$, referred to the molar volume of water in a cell (v), called water potential (ψ) and used in the study of the water regime of plants (Ondar, 2011).

$$\psi = (\mu - \mu_0)/V \qquad (9)$$

where: ψ – water potential, represents the energy that causes the phenomenon of osmosis in biological systems called pressure deficit or suction force; V – the partial molecular volume of water, $cm^3.mol^{-1}$

The water potential ψ is the algebraic sum of the individual potentials of the system components: osmotic, turgor, matrix and gravitational potentials. If two cells with different potentials ψ are located nearby, water will diffuse between them through the cell wall from a cell with a higher water potential into a cell with a lower potential.

It should be noted that the water entering the root system of plants is not pure (distilled) water, but aqueous solutions containing various mineral substances. In principle, in this situation, one should probably consider a system consisting of water and mineral substances such as: calcium, potassium, sodium, iron, magnesium, aluminium, nitrogen and many others. Depending on the composition and concentration of mineral substances, aqueous solutions will have completely other physicochemical properties and physiological effects compared to pure water (Masimov et al., 2019).

Examples of the influence of the mineral composition on the properties of mineral waters can be given. Alkaline mineral water belongs to the hydrocarbon group of water obtained from natural sources. The acidity of water exceeds pH 7. Because of the predominance of bicarbonate ions (HCO_3^-), sodium, potassium, magnesium and other minerals in it, the water is conditionally called alkaline, and its beneficial properties are used to treat several diseases.

Sulfate-chloride, sodium-calcium mineral water is distinguished by a relatively high content of iodine, bromine and fluorine with a total content of up to

5.0–5.5 g.dm^{-3}. It was noted (Masimov, 2018), that the thermodynamic state of intracellular water is significantly affected by ions, increasing, or slowing down the mobility of the water layers adjacent to them. Thus, ions Na^{+}, Ca^{2+}, Ba^{2+}, Mg^{2+}, Al^{3+}, OH^{-}, bind water (disrupt the structure of water) and are positively hydrated slowing down the mobility of water molecules. At the same time, K^{+}, NH^{4+}, Pb^{4+}, CS^{+} ions are negatively hydrated and increase water mobility. Ions with negative hydration strongly disrupt the structure of water.

The activity of water in cells depends on temperature, pressure, hydrophobic interaction with non-polar components. The hydrophobic interaction of water with non-polar components is determined by their quantity, composition and conformational changes that affect cell metabolism, the magnitude of the gradient of water activity inside and outside the cell (Mosin and Ignatov, 2011; Zemskov, 2018; Henry, 2021).

Entropy of water

At present, it has been proven that the features of the physical properties of water and numerous short-lived hydrogen bonds in a water molecule create favourable conditions for the formation of special associated molecular structures – clusters (Sidorenko and Ivanova, 2011; Brindza et al., 2014; Horčinova Sedlačkova et al., 2022). Cluster is the structural unit of structured water. Structured water (Smirnov, 2003; Nelson and Cox, 2022), consisting of many clusters of various types, forms a hierarchical spatial liquid crystal structure with special physical properties called activated water. For example, it was developed technology by the "Resonance Effect Technology – MRET", and on its basis, the water activation device was patented in the USA (Patent USA no. 6022479, 1998; Smirnov and Trongsawad, 2006). The activation process consists in changing the structure of water by creating stable dynamic multiple molecular layers of water, similar to the structures of cellular water in living organisms. Activated water retains its basic structure and a significant part of its useful properties for a day at room temperature and at least 45 days at a temperature of +4 °C.

Numerous experimental data affirm the fact that the mechanism of interaction between dissolved substances and water promotes the formation of complex molecular systems that have new physical and chemical parameters and biological activity. However, activated water obtained in various ways is not stable and loses its acquired properties within a short time.

First of all, it should be noted that water has unique properties associated to a certain extent with the polarity of its molecules and their ability to form hydrogen bonds with each other. Therefore, the electrical polarity of liquid water molecules has the property of forming a large number of hydrogen bonds with a total strength between the molecules.

At any moment in time, the vast majority of liquid water molecules are linked by hydrogen bonds. The lifetime or stability of each hydrogen bond is only 10^{-10}... 10^{-11} s. The energy of one hydrogen bond is only about 21 kJ.mol^{-1} (Wernet et al., 2004; Nelson and Cox, 2022). In liquid water, there is a constant formation and destruction of hydrogen bonds between molecules. As a consequence of this phenomenon, the structure of liquid water is dynamic, being fluid and non-fluid at the same time (Pershin, 2006; Nelson and Cox, 2022). However, almost all water molecules are constantly connected by hydrogen bonds. This structure ensures the stability of the branched structure of liquid water, water vapour, as well as frozen crystalline water (Mosin and Ignatov, 2011).

According to the second law of thermodynamics and its statistical nature, the state of various systems, including water, is observed with a high degree of precision. In biological and food systems, water is simultaneously in a macro- and microstate. The microstate is liquid, vaporous and solid (crystalline) water. A microstate is characterized by the arrangement of molecules in a medium at a given point in time. In general, the state of water is determined by the thermodynamic entropy function according to the Boltzmann formula (Rubin, 1987):

$$S = k \ln W \qquad (10)$$

where: S – entropy, J.mol^{-1}.K^{-1}; k – Boltzmann constant, represents the ratio between the universal gas constant – R, and the Avogadro number – NAV: $k = R/N_{AV}$; (R = 8.31 J.mol^{-1}.K^{-1}; k = 1.38 × 10^{23} J.K^{-1}); ln W – logarithm of the probable number of microstates of the system

In this case, the maximum number of microstates through which a given macrostate of water (liquid water) is realized is determined by the thermodynamic probability (W). The Boltzmann formula is applied to estimate the number of water microstates.

Under standard conditions, T = 298 °K, p = 1 atm, n = 1 mol, the value of the entropy of liquid water is: $S(H_2O)$ = 70 J.mol^{-1}.K^{-1}. At a concentration of water molecules

equal to NAV = 1 mol, the Boltzmann constant is: k = R J.mol^{-1}.K^{-1}. The number of microstates per 1 mol of water molecules according to formula (10) will be:

$$W = e^{S/R} = e^{70/8.31} = 4537$$

Thus, it turns out that for 6 1023 molecules of liquid water, at a standard temperature of 298 °K (25 °C), there are more than 4500 microstates of water (combinations of water molecules) with an equal probability of realizing one macrostate of liquid water.

Biological systems are open non-equilibrium thermodynamic systems. The change in entropy in biological systems is complex due to the constant exchange of energy and substances with the environment. The change in the entropy of water as an element of these systems is not equal to zero $\Delta S \neq 0$. As a result of these processes, the total change in entropy in biological living systems is always positive.

Food systems, being derivatives of biological systems, as a rule, are irreversible closed systems. Their changes are accompanied by an increase in entropy $\Delta S > 0$. As the entropy in the food system increases, the energy dissipation increases, the entropy increases to a maximum value and the thermodynamic equilibrium of the system is established. However, the change in the entropy of water in food systems is zero $\Delta S = 0$.

In principle, the change in the entropy of water in biological and food does not occur. This is one of the many signs of the unique physical and chemical properties of water. When ice is heated, a phase transition occurs: ice → water; water → vapour; vapour → water; etc. (Carrrasco et al., 2009). The change in entropy during phase transitions of water is equal to the heat of the phase transition. Thus, during the phase transitions of water, an abrupt change in entropy occurs. Under standard conditions, the entropy of ice is 48 J.mol^{-1}.K^{-1}, that of water – 70 J.mol^{-1}.K^{-1}, and that of vapour – 190 J.mol^{-1}.K^{-1} (Stepanovskikh and Brusniczyna, 2008). In reversible phase transitions of the type: water → vapour → water; water → ice → water; ice → water → vapour, no increase in entropy occurs ($\Delta S = 0$) due to the equality of the expended and released energy.

The transition of water from one state to another takes place not only in biological and food systems but is a well-known natural phenomenon. The uniqueness of this phenomenon lies in the fact that during the phase transitions of water, there are no abrupt changes in the physicochemical properties and, especially, the appearance of an anomaly. Even in biological systems, when water passes from a bound to a free state, water retains its physical and chemical properties. The innumerable number of phase transitions water → vapor → water; water → ice → water did not lead to a change in the structure of unbound water. This indicates a stationary state of unbound water, which is possible with minimal energy consumption. At the same time, liquid water does not reach an equilibrium state with the environment. Nevertheless, it is possible that the study of the structure and function of activated water, methods of obtaining it, will expand our knowledge and present us with new discoveries.

Acknowledgement

This research was performed in the framework of the ERASMUS+ program for staff mobility training in 2021–2022 and was supported by the International Visegrad Scholarship Fund through grant #52210146.

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

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Evaluation of honeys in some quality indicators obtained from different plant species and locations

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Article Details:
Received: 2022-11-13
Accepted: 2022-11-28
Available online: 2022-11-30

DOI: https://doi.org/10.15414/ainhlq.2022.0029

The publication aimed to evaluate 40 samples of honey obtained from beekeepers from different types of plants (monofloral – *Aesculus hippocastanum*, *Brassica napus*, *Fagopyrum esculentum*, *Helianthus annuus*, *Phacelia tanacetifolia*, *Robinia pseudoacacia*, *Sinapis alba*, *Tillia* spp., and multiflorous) and different locations (Donetsk, Zhytomyr, Kyiv, Kharkiv, Kherson and Ivano-Frankivsk) in Ukraine in selected quality indicators. We determined significant differences between the samples in all honey quality indicators. In the collection of honey samples, we determined Moisture in the range of 15.20–23.20%, diastase 1.10–22.26 °Goethe, hydroxymethylfurfural 1.50–29.00 mg.kg^{-1}, sugar content 66.51–98.87%, sucrose 0.23–9.61% and proline content 119.29–334.31 mg.kg^{-1}. The quality indicators of the evaluated honey samples were determined within the limits according to the established criteria of *Codex Alimentarius* and EU legislation. We determined a water content of more than 20% in three honey samples. We determined lower HMF values than 3 in 4 honey samples and higher HMF values than 1 in 3 honey samples. We determined a lower proline content than 180 mg.kg^{-1} in 16 honey samples. We determined higher values of sucrose content than 5 g.100 g^{-1} in 5 evaluated honey samples. The results confirmed significant differences between the evaluated honey samples. Some samples of evaluated honey did not reach the required criteria for honey quality.

Keywords: honey, locality, moisture, diastase, hydroxymethylfurfural, sugar, sucrose, proline

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Introduction

China is the world's largest exporter of honey, with total exports of 128,330 tons in 2016. They are followed by Argentina (81,183 tons), Ukraine (54,442 tons), Vietnam (42,224 tons), India (35,793 tons), Mexico (29,098), Spain (26,874 tons), Germany (25,325 tons), Brazil (24,203 tons), and Belgium (20,816 tons) (Raezke et al., 2018).

Honey is mainly composed of water (15–20%) and two sugars (dextrose and levulose), with the presence of small amounts of at least 22 other more complex sugars (80–85%, w/w). Honey is mainly composed of sugar components, especially fructose and glucose, followed by sucrose and maltose (Kamal and Klein, 2011). Sugar in honey is responsible for the viscosity, and the hygroscopic and granulation characteristics of the honey. However, the sugar content of the honey depends on the botanical and geographical regions (Tafere, 2021).

Honey has also been reported to contain an intricate mixture of nitrogenous compounds, lactone, proteins, antibiotic-rich inhibine, enzymes, phenol antioxidants, aroma compounds, amino and organic acids, gluconic acid, phenolic acids, flavonoids, minerals, vitamins, 5-hydroxymethylfurfural (HMF) and other phytochemicals (Gheldof et al., 2002). Honey composition varies depending on its floral, geographical and entomological sources (Anklam, 1998; Gheldof et al., 2002; Tafere, 2021).

The honey composition, colour, aroma, and flavour depend mainly on the plant species and geographical regions involved in its production, and are also affected by processing, manipulation, packaging, and storage time (Tornuk et al., 2013; Escuredo et al., 2014; Karabagias et al., 2014; Tafere, 2021; Rajindran et al., 2022).

The main quality parameters of honey are diastase activity, the concentration of proline and electrical conductivity, as well as the content of free acid, hydroxymethylfurfural (HMF) and sucrose (Thrasyvoulou et al., 2018; Council EU, 2001).

Proline is the predominant free amino acid of honey, and it is a measure of the level of total amino acids (Truzzi et al., 2014). The proline content of honey is measured as a criterion for estimating the quality (Bogdanov, 2002) and the antioxidant activity of the honey (Meda et al., 2005; Saxena and Gautam, 2010) and it may be used also for characterization based on botanical origin (Bogdanov et al., 2004).

There are more than 180 substances in honey, but it is so distinctive and helpful primarily due to the presence of enzymes, which were brought by bees during the nectar processing (Aljohar et al., 2018). Activated enzymes are very sensitive to high temperatures and will lose their activity when they exceed a certain temperature. However, concentrated honey will go through high temperatures in the process of concentration, leading to the inactivation of a large number of active substances. Therefore, it is necessary to explore the effects of different heating conditions on the activity of enzymes in the honey.

Apart from several other components, honey also contains enzymes which are responsible for converting nectar and honeydew to honey. ln honey there are α-glucosidase (invertase), α- and β-amylase (diastase), glucose oxidase, catalase and acid phosphatase. The enzyme activity in honey has been widely studied for many years (Persano Oddo et al., 1999; Bonvehi et al., 2000; Vorlová and Přidal, 2002; Belay et al., 2017).

Honey contains multiple enzymes at low concentrations, the most prominent of which are diastase, invertase (α-glucosidase), glucose-oxidase, catalase, and acid phosphatase (Sak-Bosnar and Sakač, 2012). As one of the most important enzymes, diastase (α- and β-amylase) not only enriches the nutritional and therapeutic function of honey but is also taken as an important index to evaluate honey qualities. The diastase activity is usually expressed in Schade units (Kedzierska-Matysek et al., 2016), also known as the diastase number (DN), which is defined as the amount of enzyme that will convert 0.01 g of starch to the prescribed end-point in 1h at 40 °C under the conditions of the test. According to the Honey Quality and International Regulatory Standards, the diastase activity must not be less than or equal to 8, determined after processing and blending for all retail honey, and the activity must not be less than 3 for honey with naturally low enzyme content (Huang et al., 2019).

Diastase (α-amylase) is one of the predominant enzymes in honey, next to invertase and glucose oxidase, which is added to honey by the bee during the collection and ripening of flower nectar (Persano Oddo et al., 1990). One unit of diastase activity is defined as that amount of α-amylase, which will convert 0.01 gram of starch to the prescribed end-point in one hour at 40 °C. The results are expressed in Schade units per gram of honey and termed Diastase Number (DN) (Bogdanov, 2002).

An organic compound known as 5-hydroxymethylfurfural (HMF) is formed from

reducing sugars in honey and various processed foods in acidic environments when they are heated through the Maillard reaction. In addition to processing, storage conditions affect the formation of HMF, and HMF has become a suitable indicator of honey quality. HMF is easily absorbed from food through the gastrointestinal tract and, upon being metabolized into different derivatives, is excreted *via* urine. In addition to exerting detrimental effects (mutagenic, genotoxic, organotoxic and enzyme inhibitory), HMF, which is converted to a non-excretable, genotoxic compound called 5-sulfoxymethylfurfural, is beneficial to human health by providing antioxidative, anti-allergic, anti-inflammatory, anti-hypoxic, anti-sickling, and anti-hyperuricemic effects. Therefore, HMF is a neo-forming contaminant that draws great attention from scientists (Shapla et al., 2018).

Beekeeping has been widely promoted in many countries as a major contributor to rural development. Honey is a sweet and viscous liquid which has sweetness due to the presence of monosaccharides. The major constituents of honey are sugars, water, proteins, enzymes, acids and minerals, while the major causes of quality deterioration include heating at high temperatures, high moisture content, adulteration, poor packaging and poor storage conditions.

The present work is a study of the moisture, natural occurrence of sugar content, hydroxymethylfurfural (HMF), proline and diastase in different honey samples from various regions of Ukraine.

Material and methodology

Origin of honey

The pollen analysis for identification of the botanical origin of Ukrainian kinds of honey was conducted at the laboratories of the Department of Certification and Standardization of Agricultural Products, National University of Life and Environmental Sciences (NULES) of Ukraine. They were analysed 40 samples of various kinds of honey (monofloral – *Aesculus hippocastanum*, *Brassica napus*, *Fagopyrum esculentum*, *Helianthus annuus*, *Phacelia tanacetifolia*, *Robinia pseudoacacia*, *Sinapis alba*, *Tillia* spp., and multiflorous) (Table 1).

Table 1 Basic information about evaluated honey samples

№	Origin	Region	№	Origin	Region
S01	spring grasses	Donetsk	**S21**	*Helianthus annuus*	Zhytomyr
S02	*Robinia pseudoacacia*	Donetsk	**S22**	*Helianthus annuus*	Zhytomyr
S03	multiflorous	Donetsk	**S23**	*Helianthus annuus*	Zhytomyr
S04	multiflorous	Donetsk	**S24**	*Helianthus annuus*	Zhytomyr
S05	*Helianthus annuus*	Donetsk	**S25**	*Helianthus annuus*	Zhytomyr
S06	*Robinia pseudoacacia* (commercial honey)	Ukraine	**S26**	*Helianthus annuus*	Zhytomyr
S07	*Robinia pseudoacacia* (commercial honey)	Ukraine	**S27**	multiflorous	Zhytomyr
S08	*Brassica napus*	Kyiv	**S28**	multiflorous	Zhytomyr
S09	*Robinia pseudoacacia*	Kyiv	**S29**	multiflorous	Zhytomyr
S10	*Tillia* spp.	Kyiv	**S30**	*Helianthus annuus*	Kharkiv
S11	*Robinia pseudoacacia*, *Tillia* spp., *Phacelia tanacetifolia*	Kyiv	**S31**	*Helianthus annuus*	Kharkiv
S12	*Aesculus hippocastanum*, *Robinia pseudoacacia*, *Phacelia tanacetifolia*	Zhytomyr	**S32**	*Helianthus annuus*	Kharkiv
S13	*Fagopyrum esculentum*, *Sinapis alba*, *Phacelia tanacetifolia*, *Robinia pseudoacacia*	Kyiv	**S33**	*Helianthus annuus*	Kharkiv
S14	*Tillia* spp.	Kyiv	**S34**	multiflorous (eco honey)	Ivano-Frankivsk
S15	Orchard, Forest grasses, *Robinia pseudoacacia*	Kyiv	**S35**	multiflorous (eco honey)	Ivano-Frankivsk
S16	*Robinia pseudoacacia*, *Phacelia tanacetifolia*, *Tillia* spp.	Zhytomyr	**S36**	multiflorous (eco honey)	Ivano-Frankivsk
S17	*Robinia pseudoacacia* (commercial honey)	Ukraine	**S37**	*Brassica napus*	Zhytomyr
S18	*Robinia pseudoacacia* (commercial honey)	Ukraine	**S38**	*Brassica napus*	Zhytomyr
S19	*Robinia pseudoacacia* (commercial honey)	Ukraine	**S39**	*Echium vulgare*	Kherson
S20	medicinal herbs	Kyiv	**S40**	medicinal herbs	Kyiv

Physico-chemical analysis

The analysis was conducted at the Ukrainian Laboratory of Quality and Safety of Agricultural Products.

Chemicals

All chemicals were of analytical grade and were purchased from LLC "NVP"ALFARUS" (UA).

Methods

The mass fraction of water was determined on an LR-01 laboratory refractometer (Maselli Misure s.p.a., Italy) using a standardized technique according to DSTU 4497:2005 (2007). Hydroxymethylfurfural, diastase activity, proline, the proportion of invert sugars and sucrose were investigated with a KFC-3 photo calorimeter (UA) using standardized methods according to DSTU 4497:2005 (2007).

Statistical analyses

Basic statistical analyses – the minimal and maximal values of the traits, arithmetic means, and coefficient of variation (CV, %) were performed using PAST 2.17. Results of the morphometric analysis were determined by mean ± standard deviation (SD) and statistical significance was estimated. The level of variability was determined by Stehlíková (1998). Hierarchical cluster analyses of similarity between plants were computed by the Bray-Curtis similarity index and were performed using PAST 2.17. All the observed traits were shown in graphic form.

Criteria for evaluating the quality of honey

By evaluating honey samples, we respected the honey quality criteria according to European directive 2001/110/EC and revised the *Codex* standard for honey. The *Codex* standard for honey adopted by the *Codex Alimentarius* Commission in 1981, revised in 1987 and 2001, has voluntary application and serves in many cases as a basis for national legislation (*Codex Alimentarius*, 2001b). The European Council followed the recommendations of *Codex* and issued Directive 2001/110/EC (EC, 2001), amended 2014/63/EU that laid down the production and trading parameters of honey within the Member States of the EU (EU, 2014).

Results and discussion

Natural honey is sticky, a viscous solution containing about 15–19% water, 80–85% carbohydrates (mainly glucose and fructose), 0.1–0.4% proteins, 0.2% ash and minor amounts of amino acids, enzymes, vitamins and other substances such as phenolic antioxidants (Buba et al., 2013; Kek et al., 2017; Živkov Baloš et al., 2019).

The total variability for the evaluated honey quality indicators is presented in Table 3 in the form of coefficients of variation. The results show that a low degree of variability was determined only for moisture (10.17%) and sugar content (12.31%). We noted a high degree of variability in the determined diastasis values (32.93%). We determined a very high degree of variability in HMF (52.80%). We determined an extremely high degree of variability in the sucrose content, up to 97.86%.

Moisture content

The water content (moisture) in honey depends on the production season, floral source, abundance of nectar flow, soil, ventilation of the beehive, colony strength, and meteorological conditions in the areas of honey production, primarily air humidity (Sousa et al., 2016; Lazarević et al., 2017). An important factor that could affect the water content is honey maturation and harvest time (Živkov Baloš et al., 2019).

The water content of honey (water-in-honey) is the quality aspect that determines the ability of honey to remain fresh and to avoid spoilage by yeast fermentation. Raw honey can have a water-in-honey content of less than 14% and the lower the water content the higher the perceived value of the honey. It is internationally recognized that good quality honey should be processed at less than 20% water content. Low water content is desirable because honey may begin to ferment and lose its fresh quality if the water-in-honey is greater than 20% (Tafere, 2021).

In the analysed honey samples (Tables 2 and 4) we found a moisture content in the range of 15.2 (S16 and S28 Multiflorous – Zhytomyr) – 23.2% (S31 Sunflower – Kharkiv).

Živkov Baloš et al. (2019) determined water content in different honey samples produced in regions of Serbia in the range from 15.2 ±0.8% (honeydew) to 18.9 ±1.8% (linden honey). Thrasyvoulou (1986) determined moisture 16.3–18.1% in blossom honeys and 15.3–18.3% in honeydew honeys.

All evaluated honey samples in the publications meet EU legislative criteria according to European directive 2011 and 2014 and revised *Codex* standard for honey (*Codex*, 2001; *Alimentarius*, 2001b).

Table 2 Comparison of honeys in some quality indicators obtained from different plant species and different locations in Ukraine

№	Moisture/water, %	Diastase, °Goethe	HMF, mg.kg^{-1}	Sugars, %	Sucrose, %	Proline, mg.kg^{-1}
S01	17.2 ±0.00	7.62 ±0.15	10.8 ±0.10	98.15 ±0.16	1.17 ±0.21	245.28 ±0.95
S02	15.8 ±0.00	9.11 ±0.19	10.1 ±0.10	95.95 ±0.10	0.73 ±0.10	132.69 ±0.95
S03	16.2 ±0.10	22.26 ±0.10	15.8 ±0.10	89.08 ±0.05	0.84 ±0.11	280.13 ±0.95
S04	17.0 ±0.10	10.15 ±0.05	2.3 ±0.19	88.15 ±0.10	2.13 ±0.11	237.24 ±0.80
S05	17.0 ±0.10	7.52 ±0.50	12.4 ±0.10	99.09 ±0.05	0.32 ±0.00	119.29 ±0.85
S06	17.1±0.00	12.11 ±0.15	10.5 ±0.10	92.44 ±0.09	1.04 ±0.05	298.89±0.95
S07	16.5 ±0.10	14.01 ±0.10	11.3 ±0.10	95.72±0.09	1.03 ±0.04	290.85 ±0.95
S08	17.8 ±0.10	9.45 ±0.05	10.4 ±0.19	95.69 ±0.00	1.05 ±0.05	207.75 ±0.95
S09	16.8 ±0.10	1.10 ±0.10	11.4 ±0.10	92.70 ±0.04	9.61 ±0.00	247.96 ±0.95
S10	21.0 ±0.10	12.75 ±0.00	4.2 ±0.19	88.45 ±0.14	8.89 ±0.09	148.77 ±0.85
S11	21.2 ±0.00	12.71 ±0.15	15.8 ±0.10	103.28 ±0.05	1.33 ±0.00	296.21 ±0.95
S12	18.4 ±0.00	10.12 ±0.05	11.0 ±0.00	86.27 ±0.05	7.37 ±0.14	264.04 ±0.95
S13	18.6 ±0.10	8.56 ±0.50	15.1 ±0.19	94.65 ±0.05	1.42 ±0.05	260.52 ±1.06
S14	18.6 ±0.10	12.47 ±0.05	12.0 ±0.00	96.54 ±0.04	0.80 ±0.00	176.19 ±1.06
S15	15.3 ±0.00	14.25 ±0.10	5.8 ±0.10	93.44 ±0.04	1.32 ±0.00	334.31 ±0.00
S16	15.2 ±0.00	12.13 ±0.00	14.1 ±0.10	90.95 ±0.04	0.84 ±0.04	224.38 ±1.06
S17	16.8 ±0.10	11.09 ±0.00	10.3 ±0.00	67.76 ±0.05	1.58 ±0.00	209.32 ±1.06
S18	17.0 ±0.00	8.65 ±0.15	11.4 ±0.10	77.03 ±0.05	1.48 ±0.11	254.49 ±1.06
S19	17.2 ±0.10	9.98 ±0.10	12.3 ±0.10	86.24 ±0.05	0.23 ±0.00	298.16 ±0.00
S20	18.2 ±0.10	11.36 ±0.05	13.6 ±0.19	81.44±0.00	2.42 ±0.05	169.38 ±0.00
S21	18.1 ±0.10	14.01 ±0.05	29.0 ±0.19	73.24 ±0.05	1.18 ±0.11	170.38 ±2.72
S22	17.0 ±0.10	9.84 ±0.05	23.0 ±0.19	87.94 ±0.05	1.91 ±0.00	275.25 ±1.36
S23	16.5 ±0.00	5.14 ±0.10	12.6 ±0.10	78.15 ±0.05	2.58 ±0.05	300.27 ±0.00
S24	18.8 ±0.00	12.75 ±0.00	2.6 ±0.10	72.35 ±0.05	0.65 ±0.11	216.13 ±1.48
S25	21.2 ±0.10	4.79 ±0.10	6.6 ±0.10	109.08 ±0.06	1.07 ±0.06	199.34 ±1.48
S26	20.8 ±0.10	11.20 ±0.15	6.9 ±0.19	98.55 ±0.11	1.51 ±0.06	178.36 ±1.48
S27	17.7 ±0.00	11.27 ±0.00	1.5 ±0.00	97.74 ±0.05	2.41 ±0.14	187.08 ±1.39
S28	15.2 ±0.10	11.07 ±0.10	6.0 ±0.10	78.18 ±0.21	6.25 ±0.10	218.58 ±1.39
S29	19.6 ±0.10	14.45 ±0.15	7.4 ±0.10	91.25 ±0.11	2.25 ±0.05	191.02 ±1.38
S30	18.1 ±0.10	12.56 ±0.00	3.1 ±0.19	68.91 ±0.16	0.64 ±0.11	303.63 ±1.40
S31	23.2 ±0.00	12.44 ±0.05	10.4 ±0.19	73.53 ±0.06	3.38 ±0.06	240.12 ±1.40
S32	18.8 ±0.00	10.44 ±0.05	10.1 ±0.10	78.63 ±0.05	2.38 ±0.11	174.64 ±0.00
S33	19.3 ±0.10	11.28 ±0.00	9.5 ±0.0	81.08 ±0.05	2.40 ±0.00	175.64 ±2.81
S34	18.8 ±0.10	8.60 ±0.10	8.0 ±0.10	66.51 ±0.05	1.84 ±0.00	283.78 ±1.40
S35	21.4 ±0.00	20.60 ±0.15	6.7 ±0.00	76.09 ±0.06	1.01 ±0.11	277.33 ±0.00
S36	20.0 ±0.00	7.88 ±0.10	8.8 ±0.00	81.02 ±0.05	3.79 ±0.05	183.11±1.26
S37	18.1 ±0.10	11.25 ±0.05	7.6 ±0.10	81.71 ±0.05	1.29 ±0.00	168.89 ±1.26
S38	18.8 ±0.00	12.31±0.00	6.5 ±0.10	87.94 ±0.05	7.47 ±0.00	151.11 ±1.26
S39	17.4 ±0.10	14.48 ±0.05	6.0 ±0.10	92.88 ±0.00	2.02 ±0.00	169.89 ±1.26
S40	17.3 ±0.10	11.10 ±0.05	5.8 ±0.10	69.34 ±0.05	2.66 ±0.00	152.89 ±0.00

Notes: HMF – hydroxymethylfurfural

Table 3 Basic statistical characteristic of the variability of evaluated honey samples

Indicator	Moisture, %	Diastase, °Goethe	HMF, mg.kg^{-1}	Sugars, %	Sucrose, %	Proline, mg.kg^{-1}
Min	15.20	1.10	1.50	66.51	0.23	119.29
Max	23.20	22.26	29.00	109.08	9.61	334.31
x̄	18.13	11.12	9.97	86.43	2.36	222.83
s	1.84	3.66	5.26	10.64	2.31	56.43
V, %	10.17	32.93	52.80	12.31	97.86	25.32

Notes: HMF – hydroxymethylfurfural (mg.kg^{-1}); min, max – minimal and maximal measured values; x̄ – arithmetic mean; s – standard deviation; V – coefficient of variation (%)

Sugars content

Honey is a sweet, thick, supersaturated sugar solution produced by honey bees (*Apis mellifera*) from plant nectars, plant secretion and excretions of plant-suckling insects of the living parts of plants (*Codex Alimentarius*, 2001a). It is one of the known natural sources of sweetness and energy for man. Honey is composed mainly of disaccharides which contain two monosaccharides, glucose and fructose, with a percentage of water and other groups of substances (Kamal and Klein, 2011). Small quantities of other sugars are also present, in the form of other disaccharides, trisaccharides and oligosaccharides which are formed during the ripening and storage effects of bee enzymes and acids of honey (Ball, 2007). Chemical compositions of honey differ depending on the plant species on which the bees forage, the climatic conditions, and other factors (Buba et al., 2013). The very concentrated solution of several sugars produces the characteristic physical properties of honey like high viscosity, high density, graduation tendencies, tendency to absorb water from the atmosphere and immunity from some types of spoilage.

More than 95% of the honey solids are carbohydrates, with monosaccharides (fructose and glucose) predominating. The presence of monosaccharides (fructose, glucose), disaccharides (e.g. maltose, sucrose, isomaltose,), and oligosaccharides (e.g. erlose, melezitose, raffinose) in most abundantly produced and, on the other hand, also in very specific honeys is documented (Cote et al., 2003; De La Fuente et al., 2006; Ouchemoukh et al., 2010; Pacholczyk-Sienicka et al., 2022).

Sugars represent the largest portion of honey composition (i.e., more than 95% of the honey solids); the monosaccharides fructose and glucose are the most abundant while small amounts of disaccharides (maltose and sucrose) are also present; other disaccharides and higher sugars (trisaccharides and oligosaccharides) are also present in quite small quantities. Due to the high content of monosaccharides (fructose and glucose) and relatively low moisture content, the water activity of honey is usually, but not always, below 0.60 which is enough to inhibit the growth of osmotolerant yeasts (Zamora and Chirife, 2004; Chirife et al., 2006).

Generally, honey is rich in glucose and fructose and the percentage of sucrose in honey should be lower, which is less than 5% (*Codex Alimentarius*, 2001a). However, it is assumed that green honey contains higher sucrose content compared to glucose and fructose level (Rajindran et al., 2022).

In the evaluated honey collections, we determined the sugar content in the range from 66.51% (S34 Multiflorous – Ivano-Frankivsk) to 98.78% (S25 Sunflower – Zhytomyr). Bandeira et al. (2018) determined in the honey collection the content of sugars in the range of 62.87–91.56%.

Sucrose content

The general provision for sucrose content is less than 5% with the exception listed for both *Codex* and Directive. From these exceptions, only *Eucalyptus*, *Robinia*, *Citrus* and *Lavandula* are listed as important for honey production and can be found predominantly in honey. The sucrose content of honey from *Eucalyptus* generally is less than 4.2% (Persano Oddo and Piro, 2004) while honey from dandelion (*Taraxacum officinale*) may occasionally have sucrose of more than 5%.

Directive 2001/110 EU declares the following Compositional criteria for honey.

Sucrose content:

- in general, not more than 5 g.100 g^{-1};
- false acacia (*Robinia pseudoacacia*), alfalfa (*Medicago sativa*), Menzies Banksia (*Banksia menziesii*), French honeysuckle (*Hedysarum*), red gum (*Eucalyptus camadulensis*), leatherwood (*Eucryphia lucida*, *Eucryphia milliganii*), *Citrus* spp. not more than 10 g.100 g^{-1};
- lavender (*Lavandula* spp.), borage (*Borago officinalis*) not more than 15 g.100 g^{-1}.

Sucrose reached a value in our honey samples (Tables 2 and 4) the contents were in the range of 0.23 (S19 *Robinia pseudoacacia* (commercial honey) – Ukraine to 9.61 (S09 *Robinia pseudoacacia* – Kyiv). We determined higher values of sucrose content than 5 g.100 g^{-1} in 5 evaluated honey samples, namely S09 (*Robinia pseudoacacia* – Kyiv), S10 (*Tillia* spp. – Kyiv), S12 (Multiflorous – Zhytomyr), S28 (Multiflorous – Zhytomyr) and S38 (*Brassica napus* – Zhytomyr).

Thrasyvoulou (1986) determined sucrose in the interval 1.5–4.2 g.100 g^{-1} in blossom honeys and 5.6–7.2 g.100 g^{-1} in honeydew honeys.

Tarapatskyy et al. (2021) studied botanical origin of Polish honey based on physicochemical properties and bioactive components. Authors determined sucrose content in linden (3.22–5.08 g.100 g^{-1}), buckwheat (0.35–0.67 g.100 g^{-1}), honeydew (5.17–10.46 g.100 g^{-1}), and multifloral honey (4.02–6.78 g.100 g^{-1}).

Hydroxymethylfurfural (HMF) and diastase

HMF is a breakdown product of sugars, produced when honey is heated, and diastase is an enzyme that is inactivated by heating. The levels of these two constituents also change during storage. Because the rate of HMF formation and diastase inactivation during storage or heating varies in different honeys, and also because there is a large variation in amounts of them in fresh, unprocessed honeys, doubts have arisen about the validity of their use as evidence of overheating (Schade et al., 1958).

Directive 2001/110 EU (EC, 2001) declares the following Compositional criteria for honey.

Diastase activity and hydroxymethylfurfural content (HMF) determined after processing and blending:

a) Diastase activity (schade scale):
   - in general, except baker's honey not less than 8;
   - honeys with low natural enzyme content (e.g., citrus honeys) and HMF content of not more than 15 mg.kg^{-1}, not less than 3 mg.kg^{-1}.

b) HMF
   - in general, except baker's honey, not more than 40 mg.kg^{-1} (subject to the provisions of (a), second indent);
   - honeys of declared origin from regions with tropical climates and blends of these honeys.

In our honey samples (Tables 2 and 4) the diastase content observed values from 1.10 (S9 *Robinia pseudoacacia* – Kyiv) to 22.26 (S3 Multiflorous – Donetsk) °Goethe. In the evaluated honey collection, we determined lower values as provided for by the Criteria for samples S01 (Spring grasses – Donetsk); S05 (*Helianthus annuus* – Donetsk), S09 (*Robinia pseudoacacia* – Kyiv), S23 (*Helianthus annuus* – Zhytomyr), S25 (*Helianthus annuus* – Zhytomyr) and S36 (Multiflorous – eco honey – Ivano-Frankivsk).

Hydroxymethylfurfural (HMF) reached the value in our samples (Table 2–4), the results were in the range of 1.5 (S27 Multiflorous – Zhytomyr) to 29.0 (S21 *Helianthus annuus* – Zhytomyr). In the evaluated honey collection, we recorded lower HMF values than 3 in samples S04 (Multiflorous – Donetsk), S24 (*Helianthus annuus* – Zhytomyr), S27 (Multiflorous – Zhytomyr) and S30 (*Helianthus annuus* – Kharkiv). We determined higher

Table 4 Honey samples from the evaluated collection with high and low values of the evaluated characters

Indicator/sequence of sample		High values (V) / № of sample (S)					Low values (V)/№ of sample (S)				
		1st	2nd	3rd	4th	5th	36th	37th	38th	39th	40th
Moisture, %	V	23.2	21.4	21.2	21.2	21.0	16.2	15.8	15.3	15.2	15.2
	S	31	35	25	11	10	3	2	15	28	16
Diastase, °Goethe	V	22.26	20.60	14.48	14.45	14.25	7.62	7.52	5.14	4.79	1.10
	S	3	35	39	29	15	1	5	23	25	9
HMF, mg.kg^{-1}	V	29.0	23.0	15.8	15.8	15.1	4.2	3.1	2.6	2.3	1.5
	S	21	22	11	3	13	10	30	24	4	27
Sugars, %	V	98.87	97.28	99.09	98.55	98.15	72.35	69.34	68.91	67.76	66.51
	S	25	11	5	26	1	24	40	30	17	34
Sucrose, %	V	9.61	8.89	7.47	7.37	6.25	0.73	0.65	0.64	0.32	0.23
	S	9	10	38	12	28	2	24	30	5	19
Proline, mg.kg^{-1}	V	334.31	303.63	300.27	298.89	298.16	152.89	151.11	148.77	132.69	119.29
	S	15	30	23	6	19	40	38	10	2	5

Note: S – № of sample; V – sample value in evaluated indicator; HMF – hydroxymethylfurfural

HMF values than 15 in samples S11 (Multiflorous – Kyiv), S21 (*Helianthus annuus* – Zhytomyr) and S22 (*Helianthus annuus* – Zhytomyr).

Thrasyvoulou (1986) determined diastase in the interval 27.0–60.0 DU in blossom honeys and 26.7–32.0 DU in honeydew honeys, content of HMF was not find in both honeys.

Vorlová and Přidal (2002) studied invertase and diastase activity, IN/DN ratio and HMF in fresh (floral, honeydew, compound) honeys and determined values ranged from 0.8–20.4 (IN), 11.2–30.3 (DN), 0.05–0.91 (IN/DN ratio) and 0.00–15.40 mg.kg^{-1} HMF for floral honey, 4.0–25.9 (IN), 15.9–40.3 (DN), 0.20–0.85 (IN/DN ratio) and 1.40–10.30 mg.kg^{-1} HMF for compound honey and 10.8–24.6 (IN), 13.6–45.4 (DN), 0.54–1.44 (IN/DN ratio) and 0.00–11.30 mg.kg^{-1} HMF for honeydew honey. The relation of both enzymes is expressed by the correlation r = 0.7492, p <0.01).

Tosi et al. (2008) examined treated honey samples. DN decrease from 25.8 to 8.1 after 1200 s at 90 °C heating and HMF with an initial concentration of 5.8 increased to 32.4 mg.kg^{-1} but did not reach the 60 mg.kg^{-1} limit.

Four of the most abundant honey types produced in Croatia (black locust, sage, chestnut, and honeydew honey) are characterised according to the protein and proline content and enzyme activities (Flanjak et al., 2016). The characterisation was done to determine specificities and contribute to the characterisation of unifloral honeys. Dark honey types (honeydew and chestnut honey) had a higher proline content (493.7 ±223.3 and 699.0 ±142.9 mg.1,000 g^{-1}, respectively), diastase (21.7 ±8.4 and 25.8 ±5.9 DN, respectively), and invertase (176.1 ±48.9 and 155.2 ±39.7 U.kg^{-1}, respectively) than sage and black locust honey (346.3 ±139.3 and 157.0 ±21.5 mg.1,000 g^{-1}; 19.9 ±6.8 and 11.2 ±2.1 DN; 94.7 ±52.1 and 52.1 ±20.7 U.kg^{-1}, respectively). Honeydew honey, otherwise known to possess high proline content (493.7 ±223.3 mg.1,000 g^{-1}) and enzyme activity, had a low protein content (59.4 ±21.8 mg.100 g^{-1}) comparable to black locust honey (30.4 ±7.9 mg.100 g^{-1}).

Kuc et al. (2017) studied the diastase activity of several varieties of honeys (multiflorous, honeydew and buckwheat) from different sources and stored under different conditions. Diastase activity (DN) determined by method, which is based on the distribution of the starch by α-amylase was in the range 10.9 (buckwheat and honeydew honey stored for 2 years) – 23.9 (multiflorous non-commercial from Poland, stored for 2 months at 4 °C) and results obtained by Phadebas method using UV-Spectrophotometer were in the interval 9.0 (multiflorous commercial from EU and non-EU, stored for 4 years) – 20.3 (multiflorous non-commercial from Poland, stored for 2 months at 4 °C).

The proline content

The values of proline content exceed the content of other amino acids. Its content is from 50 to 85% of the total amount of amino acids (Anklam, 1998; Hermosín et al., 2003). Proline content is a good marker of the botanical and geographical origin of honey (Costa et al., 1999). The higher content of proline is mainly found in sunflower honey, and the lower content is found in agave and eucalyptus honey. Based on the content of proline and phenylalanine, evidence of the addition of inverted syrup is possible (Singhal et al., 1997). The harmonized methods of the European Commission for honey include the spectrophotometric method for determining the proline content. A proline content lower than 180 mg.kg^{-1} may indicate the falsification of honey with the addition of sugar (Von Der Ohe et al., 1991).

The proline content in our honey samples (Tables 2 and 4) was in the range of 119.29 mg.kg^{-1} (S5 *Helianthus annuus* – Donetsk) to 334.31 mg.kg^{-1} (S15 Multiflorous – Kyiv). We determined a lower proline content than 180 mg.kg^{-1} in the following samples S01 (Spring grasses – Donetsk), S05 (*Helianthus annuus* – Donetsk), S10, S14 (*Tillia* spp. – Kyiv), S20 (Medicinal herbs – Kyiv), S21, S25, S26 (*Helianthus annuus* – Zhytomyr), S27 (Multiflorous – Zhytomyr), S32, S33 (*Helianthus annuus* – Kharkiv), S36 (Multiflorous – Eco honey – Ivano-Frankivsk), S37, S38 (*Brassica napus* – Zhytomyr), S39 (*Echium vulgare* – Kherson) and S40 (Medicinal herbs – Kyiv).

Janiszewska et al. (2012) investigated the free amino acids composition of 18 unifloral Polish honeys with different botanical origins (dominant buckwheat, raspberry, acacia, heather and goldenrod and honeydew honeys). Considerable variation in the total content of free amino acids ranging from 186.19 to 921.08 mg.kg^{-1} was stated. The dominant free amino acid in all types of honey was proline with the highest detected amount in one sample of heather honey 387.88 mg.kg^{-1}.

The proline content of Hungarian honey samples presented Czipa et al. (2012). The lowest proline concentration was measured in acacia honeys (252 ±38 mg.kg^{-1}) and the highest amount in coriander honey (2283 ±128 mg.kg^{-1}) and honeydew honey (1,089 ±137 mg.kg^{-1}). The rape, wild garlic, and asclepias honeys with values of 377 ±60 mg.kg^{-1},

476 ±27 mg.kg^{-1} and 485 ±114 mg.kg^{-1} of proline were following the acacia honeys. In the other honey types, the proline content is higher than 500 mg.kg^{-1} (linden, sunflower, chestnut, lavender). In flower honeys the proline content changed on a wide range because in these honeys the nectar and pollen ratios are very different.

Wen et al. (2017) studied and determined proline content in different floral origins (rapeseed, sunflower, buckwheat and *Codonopsis* honeys) from five different regions of China. The proline content varied among the four types of honeys, with the values decreasing in the order: buckwheat > *Codonopsis* > sunflower > rapeseed. The buckwheat honeys exhibited the highest proline content (average 610.16 mg.kg^{-1}) ($p < 0.05$), followed by *Codonopsis* honeys (494.49 mg.kg^{-1}), sunflower honeys (400.75 mg.kg^{-1}), and rapeseed honeys (201.61 mg.kg^{-1}).

In Beykaya's (2021) study, 60 honey samples (cotton, citrus, *Astragalus*, lavender, Jerusalem thorn, flower, cedarwood, pine, chestnut and *Nigella sativa*) were collected from different locations in Turkey and determined their physicochemical properties like hydroxymethylfurfural (HMF), proline, sugar content, invertase, diastase number, moisture, acidity, colour and electric conductivity (EC). The acid amounts of honeys ranged between 13.0–34.0 meq.kg^{-1} (*Astragalus* and *Nigella sativa*, respectively). The proline content of the honey samples used in this study varied between 300.0 ±11.8 and 881.7 ±42.6 mg.kg^{-1} (citrus and *Nigella sativa*, respectively) and the HMF content varied between 2.5 ±0.07 and 12.3 ±0.09 mg.kg^{-1} (cotton and cedarwood, respectively) according to honey types. Enzymes are one of the quality criteria for raw honey. The diastase number of honey samples was determined between 6.35 ±0.3 and 20.0 ±0.9 DN (citrus and *Nigella sativa*, respectively) and the amount of invertase enzyme ranged from 103.3 ±4.8 to 378.1 ±15.6 U.kg^{-1} (Jerusalem thorn and multiflorous honey).

From the evaluated collection of honey samples, we noted repeatability in groups with extreme values (Table 4) sample S05 (*Helianthus annuus* – Donetsk) 4 times (1 High – Sugars/3Low – Diastase, Sucrose and Proline), S30 (*Helianthus annuus* – Kharkiv) 4 times (1High – Proline/3 Low – HMF, Sugars and Sucrose), S02 (*Robinia pseudoacacia* – Donetsk) 3 times (3 Low – Moisture, Sucrose and Proline),

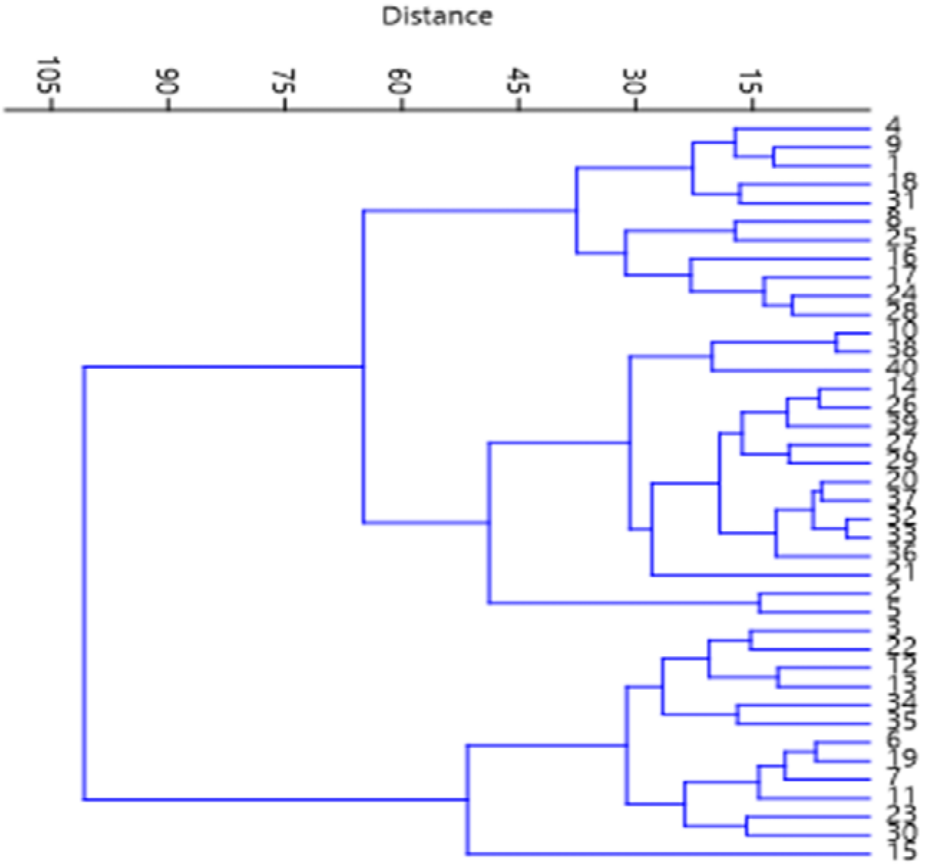

Figure 1 Cluster dendrogram of the relationships of evaluated honey samples of various origins according to some selected indicators

S10 (Multiflorous – Kyiv) 3 times (2 High – Moisture, Diastase, and 1 Low – Proline), S11 (*Tillia* spp. – Kyiv) 3 times (3 High – Moisture, HMF and Sugars) and S15 (Multiflorous – Kyiv) 3 times (3 Low – Moisture, Diastase, Proline).

Based on cluster analysis, the relationships of the tested honey samples of various origins and from different locations are graphically displayed on a dendrogram (Figure 1). The figure shows that the collection of honey was divided into 3 main clusters, which are similar in terms of their values and evaluated honey quality indicators.

Conclusion

In the presented study, 40 samples of honey obtained from beekeepers from different locations in Ukraine and different types of plants were evaluated. The collection of honey samples was evaluated in six basic indicators of honey quality – moisture, diastase, HMF, sugars, sucrose and content of proline. The obtained results expressly confirmed significant differences in each honey quality indicator as well as in the comprehensive evaluation of the samples. When comparing the results with the criteria of the standards of *Codex Alimentarius* and the EU legislation for honey, it was determined that some honey samples exceeded the specified limits. This means that not all honey samples reach the criteria for honey quality. Only 6 indicators were evaluated in the work, and such important indicators of honey quality as the content of heavy metals, the content of antibiotics, the content of residues after agro-pesticides and many others were not evaluated.Mandatory legislative control of the quality of honey for every beekeeper is not ensured in any country. Simultaneously, consumers can buy honey directly from beekeepers or supermarkets. However, it is generally known that even in supermarkets low-quality honey is provided to customers, as evidenced by the results of numerous inspections. This problem is very difficult to solve, especially if consumers buy honey directly from beekeepers. For this reason, it would be appropriate for individual countries to adopt laws for mandatory honey quality control for all beekeepers who ensure the sale of honey to consumers.

Ethical statements

This article does not contain any studies that would require an ethical statement.

Conflict of interest

None declared.

Funding

Co-author is grateful to Visegrad Fund for the scholarship for the research during which the presented knowledge was obtained.

Acknowledgements

The publication was prepared with the active participation of researchers in the International Network AgroBioNet, as a part of the international program "Agricultural Biodiversity to Improve Nutrition, Health and Quality of Life". The authors are indebted to the departmental colleagues for their support in the development of this manuscript.

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

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In vitro antioxidant response of the equine blood treated by leaf extract of *Ficus drupacea* Thunb.

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Article Details:
Received: 2022-10-14
Accepted: 2022-11-18
Available online: 2022-11-30

DOI: https://doi.org/10.15414/ainhlq.2022.0030

The present study aimed to evaluate the antioxidant potential of the aqueous extract derived from the leaves of *Ficus drupacea* Thunb. using oxidative stress biomarkers (2-thiobarbituric acid reactive substances (TBARS), aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity) and antioxidant defences (activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase GPx), ceruloplasmin (CP)) on the model of equine erythrocytes and plasma after incubation *in vitro*. Freshly collected leaves were washed, weighed, crushed, and homogenized in 0.1 M phosphate buffer (pH 7.4) (in the proportion of 1:19, w/w). The equine erythrocytes and plasma were used in the current study. A volume of 0.1 ml of the *F. drupacea* extract was added to 1.9 ml of equine erythrocytes or plasma. For positive control (blank), 0.1 ml of phosphate buffer was used. The treatment of equine plasma and erythrocytes by extract derived from leaves of *F. drupacea* resulted in reduced lipid peroxidation and oxidatively modified protein. Treatment by extract resulted in a reduced erythrocyte TBARS level of 21.9% ($p = 0.017$) compared to the untreated samples. The levels of aldehydic and ketonic derivatives of oxidatively modified proteins were non-significantly decreased. The incubation of equine plasma with an extract derived from leaves of *F. drupacea* increased antioxidant defences. The activity of SOD and GPx were increased by 41.6% ($p = 0.000$) and 61.5% ($p = 0.000$) in the equine plasma after *in vitro* incubation with an extract derived from leaves of *F. drupacea* compared to the untreated samples. The level of total antioxidant capacity was non-significantly increased. However, further detailed investigation, especially *in vivo* and *in vitro* antioxidant studies is needed to justify the use of extract derived from leaves of *F. drupacea* as a natural source of antioxidants.

Keywords: leaf extract, equine erythrocytes and plasma, lipid peroxidation, oxidatively modified proteins, total antioxidant capacity

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Introduction

Ficus drupacea Thunb. (syn. *F. mysorensis*) is a monoecious evergreen tree growing to a height of up to 35 m, hemi-epiphytic or terrestrial, with glabrous to pale or rusty brown hairy leafy twigs, which naturally occurs in SE Asia to Australia and Solomon Islands. The leaves are 10–35 cm long and 4–16 cm wide, spirally arranged or subdistichous, coriaceous, elliptic to oblong or obovate with short-acuminate apex and cordate or rounded base. The lamina is glabrous to sparsely or densely brown tomentose or woolly mainly on the large veins. Figs are axillary, in pairs or solitary, sessile and ellipsoid, 2–3 cm in diameter and up to 4.0–4.5 cm long, glabrous, at maturity yellow to orange (Berg and Corner, 2005).

The leaves of *F. drupacea* are often used to treat malaria, paragonimiasis, nasosinusitis, sinusitis, andanasarca (Kiem et al., 2013). In the screening project carried out by Kiem et al. (2013) for α-glucosidase inhibition from natural sources, these researchers found *F. drupacea* to possess an α-glucosidase inhibitory effect with 39% at a concentration of 100 $\mu g.mL^{-1}$ (Kiem et al., 2013). Also, Manjuprasanna et al. (2021) tested 29 latices from the *Ficus* genus and revealed that *F. drupacea* exhibited potent pro-coagulant and thrombin-like activity. Drupin, a thrombin-like cysteine protease responsible for platelet aggregation was purified from *F. drupacea* latex. Drupin exhibits pro-coagulant activity and reduces the bleeding time in mice tails. It induces platelet aggregation by activating mitogen-activated protein kinases and the nuclear factor-κB and PI3K/Akt signaling cascade, which, in turn, phosphorylets, cytosolic phospholipase A2 leading to the release of thromboxane A2 from the granules to activate the nearby platelets to aggregate. The results of Manjuprasanna et al. (2021) confirmed that the drupin-induced platelet aggregation was mediated by both PAR1 and PAR4, synergistically. Overall, drupin reduces the bleeding time by exerting pro-coagulant activity and induces platelet aggregation by activating the intracellular signaling cascade (Manjuprasanna et al., 2021).

The study by Manjuprasanna et al. (2020) highlights the interference of drupin in wound healing by increased arginase 1 activity and collagen synthesis, and cell proliferation and migration. These authors revealed that cysteine protease is responsible for fibrinolysis purified from the *F. drupacea* latex named drupin, and tested for its wound healing efficacy. The accelerated wound healing was mediated by the downregulation of matrix metalloprotease (MMP)-9 without altering MMP-8 expression. Besides, drupin enhanced the rate of collagen synthesis at the wound site by increasing arginase 1 activity. And also, drupin increased the expression of arginase 1 in macrophages and was involved in cell proliferation and migration *via* MAP kinase and PI3K/Akt pathways (Manjuprasanna et al., 2020).

In our previous study (Tkachenko et al., 2018, 2019), we highlight the antioxidant potential of an aqueous extract derived from leaves of other *Ficus* species using an equine erythrocyte suspension. In the study (Tkachenko et al., 2018), we have focused on the antioxidant effect of an extract derived from leaves of *F. religiosa* L. on oxidative stress biomarkers (2-thiobarbituric acid reactive substances (TBARS), carbonyl derivatives of protein oxidative modification (OMP), total antioxidant capacity (TAC)) using the model of equine erythrocytes. Treatment by extract reduced the erythrocyte's TBARS level by 25.3% ($p = 0.009$), while plasma TBARS level was increased by 75.6% ($p = 0.000$), as compared to untreated erythrocytes. When equine plasma was incubated with extract, the level of ketonic derivatives was significantly increased by 22.8% ($p = 0.000$), while a non-significantly decrease in both aldehydic and ketonic derivatives of OMP was observed (by 1.6% and 8.9%, $p > 0.05$). Treatment by *F. religiosa* extract caused the increase of TAC in plasma and erythrocyte suspension when compared to untreated erythrocytes. However, these changes were statistically non-significant. All these data suggest that *F. religiosa* could be explored for its antioxidant potential using an equine erythrocyte suspension (Tkachenko et al., 2018).

Later, we investigated the *in vitro* antioxidant activity of aqueous extracts derived from the leaves developed on the shoots of various developmental stages (juvenile and mature/generative) of *F. pumila* L. using the oxidative stress biomarkers (TBARS, carbonyl derivatives of protein oxidative modification, total antioxidant capacity) on the model of equine erythrocyte suspension (Tkachenko et al., 2019). The treatment with the extract derived from leaves of mature shoots reduced the erythrocyte's TBARS level by 22% ($p = 0.029$), while the TBARS level was increased by 15.5% ($p > 0.05$) when incubated with an extract derived from leaves of juvenile shoots as compared to untreated erythrocytes. When equine erythrocytes were incubated with the extract obtained from leaves of mature shoots, the ketonic derivatives level was significantly decreased by 6.9% ($p = 0.040$), while a non-significantly decrease in both aldehydic and ketonic derivatives of OMP was observed after

incubation with an extract derived from juvenile shoots (by 8.18 and 12.5%, p >0.05). The treatment by *F. pumila* leaf extract (from juvenile and mature shoots) caused the increase of TAC in erythrocyte suspension as compared to untreated erythrocytes. Thus, extracts derived from both juvenile and mature shoots increased the total antioxidant capacity of equine erythrocytes (Tkachenko et al., 2019).

The current study was designed to investigate the oxidative stress biomarkers (2-thiobarbituric acid reactive substances, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity) and antioxidant defences (activity of superoxide dismutase, catalase, glutathione peroxidase, ceruloplasmin) using the model of equine erythrocytes and plasma to evaluate the antioxidant activities of the aqueous extract derived from leaves of *Ficus drupacea*.

Material and methodology

Collection of plant materials

The leaves of *F. drupacea* were collected in M.M. Gryshko National Botanical Garden (Kyiv, Ukraine) and the Botanical Garden of Ivan Franko National University in Lviv (Lviv, Ukraine) in September 2016 (Figure 1). The whole collection of tropical and subtropical plants at these institutions (including *Ficus* spp. plants) has the status of a National Heritage Collection of Ukraine. Plant samples were thoroughly washed to remove all the attached material and used to prepare extracts.

Preparation of plant extracts

Freshly collected leaves were washed, weighed, crushed, and homogenized in 0.1 M phosphate buffer (pH 7.4) (in the proportion of 1:19, w/w) at room temperature. The extracts were then filtered and used for analysis. All extracts were stored at -25 °C until use. All biochemical assays were conducted at the Department of Biology, Institute of Biology and Earth Sciences, Pomeranian University in Słupsk (Poland).

Horses

Eighteen clinically healthy adult horses from the central Pomeranian region in Poland (village Strzelinko, N 54º 30’ 48.0” E 16º 57’ 44.9”), aged 8.9 ±1.3 years old, including 6 Hucul ponies, 5 Thoroughbred horses, 2 Anglo-Arabian horses and 5 horses of unknown breed, were used in this study. All horses participated in recreational horseback riding. Horses were housed in individual boxes, with feeding (hay and oat) provided twice a day, at 08.00 and 18.00 h, and water available *ad libitum*. Before sampling, all horses were thoroughly examined clinically by a veterinarian and screened for haematological, biochemical, and vital parameters, which were within reference ranges. The females were non-pregnant.

Collection of blood samples

Blood samples were collected in the morning, 90 minutes after feeding, while the horses were in the stables (between 8:30 and 10 AM) by jugular venipuncture into tubes with sodium citrate as the anticoagulant and held on the ice until centrifugation at 3,000 rpm for 5 min to remove plasma. The pellet of blood was resuspended in 4 mM phosphate buffer (pH 7.4). A volume of 0.1 ml of the plant extract was added to 1.9 ml of clean equine erythrocytes or plasma (at the final dose of an extract of 5 mg per mL). For positive control, 0.1 ml of phosphate buffer added to erythrocytes or plasma was used. After incubation of the mixture at 37 °C for 60 min with continuous stirring, biochemical assays were done. Erythrocytes and plasma aliquots were used in the study.

The 2-Thiobarbituric acid reactive substances (TBARS) assay

The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reacting substances (TBARS) with the Kamyshnikov (2004) method for determining the malonic dialdehyde (MDA) concentration. This method is based on the reaction of the degradation of the lipid peroxidation product, MDA, with 2-thiobarbituric acid (TBA) under high temperature and acidity to generate a coloured adduct that is measured spectrophotometrically. The nmol of per 1 ml was calculated using $1.56 \cdot 10^5$ mM^{-1}. cm^{-1} as the extinction coefficient.

The carbonyl derivatives of oxidative modification of proteins (OMP) assay

To evaluate the protective effects of the extract against free radical-induced protein damage in equine erythrocytes, a carbonyl derivatives content of protein oxidative modification (OMP) assay based on the spectrophotometric measurement of aldehydic and ketonic derivatives in the samples was performed. The rate of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives of amino acid reaction with 2,4-dinitrophenylhydrazine (DNFH) as described by Levine et al. (1990) and as modified by Dubinina et al. (1995). DNFH was used

Figure 1 General view of *Ficus drupacea* Thunb. specimen (A, C) and leaves of *F. drupacea* (B, D).
Photo: Yevhen Sosnovsky

for determining carbonyl derivatives in soluble and insoluble proteins. Carbonyl groups were determined spectrophotometrically from the difference in absorbance at 370 nm (aldehydic derivatives, OMP_{370}) and 430 nm (ketonic derivatives, OMP_{430}).

Measurement of total antioxidant capacity (TAC)

The TAC level in samples was estimated by measuring the 2-thiobarbituric acid reactive substances (TBARS) level after Tween 80 oxidation. This level was determined spectrophotometrically at 532 nm (Galaktionova et al., 1998). The sample inhibits the Fe^{2+}/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. The level of TAC in the sample (%) was calculated concerning the absorbance of the blank sample.

Superoxide dismutase activity assay

The activity of superoxide dismutase (SOD, E.C. 1.15.1.1) was assessed by its ability to dismutate superoxide generated in the process of quercetin auto-oxidation in an alkaline medium (pH 10.0), as proposed by Kostiuk et al. (1990). The activity was expressed in units of SOD per mL.

Catalase activity assay

The activity of catalase (CAT, E.C. 1.11.1.6) was determined by measurement of the decrease in H_2O_2 in the reaction mixture, using a spectrophotometer at the wavelength of 410 nm and the method described by Koroliuk and co-workers (1988). One unit of catalase activity was defined as the amount of enzyme necessary to decompose 1 μmol H_2O_2 per min per mL.

Glutathione peroxidase activity assay

The activity of glutathione peroxidase (GPx, EC 1.11.1.9) was determined by detecting the nonenzymatic utilization of GSH (reacting substrate) at an absorbance of 412 nm after incubation with 5,5-dithiobis-2-nitrobenzoic acid (DTNB), as proposed by Moin (1986). GPx activity is expressed as μmol GSH per min per mL.

Ceruloplasmin level assay

Ceruloplasmin (CP, E.C. 1.16.3.1) level in the plasma was measured spectrophotometrically at the wavelength of 540 nm as described by Ravin (1961). The assay mixture contained 0.1 mL of plasma, 5 mL of 0.4 M sodium acetate buffer (pH 5.5), and 0.1 mL of 0.5% *p*-phenylenediamine. The mixture was incubated at 37 °C for 60 min. Before cooling at 4 °C for 30 min, the mixture was added to 3% sodium fluoride for inhibition. Ceruloplasmin is expressed as milligrams per dL of plasma.

Statistical analysis

The mean ± S.E.M. values were calculated for each group to determine the significance of the intergroup difference. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors test ($p > 0.05$). The significance of differences between the total antioxidant capacity level (significance level, $p < 0.05$) was examined using the Mann-Whitney *U* test (Zar, 1999). In addition, the relationships between oxidative stress biomarkers were evaluated using Spearman's correlation analysis. All statistical calculations were performed on separate data from each individual with Statistica 13.3 software (TIBCO Software Inc., Krakow, Poland).

Results and discussion

The TBARS content as a biomarker of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins, and the total antioxidant capacity (TAC) in the equine erythrocytes after *in vitro* incubation with an extract derived from leaves of *F.drupacea* was assessed and shown in Figure 2.

Lipid peroxidation by reactive oxygen species (ROS) is known to be involved in the damaging mechanism of cell disorders. The most prominent and currently used assay as an index for lipid peroxidation products is the 2-thiobarbituric acid assay (TBARS test). It is based on the reactivity of an end product of lipid peroxidation, malonic dialdehyde (MDA) with 2-thiobarbituric acid to produce a red adduct (Garcia et al., 2005). As can be seen in Figure 2, treatment by extract resulted in a reduced erythrocyte TBARS level of (28.04 ±2.43 $nmol.mL^{-1}$) compared to the untreated samples (35.88 ±3.02 $nmol.mL^{-1}$). The decrease in TBARS level was by 21.9% ($p = 0.017$) (Figure 2).

Proteins are major targets for oxidation reactions (Kehm et al., 2021). As proteins are highly abundant in cells, extracellular tissues, and body fluids and react rapidly with many oxidants, they are highly susceptible to and are major targets of, oxidative damage (Hawkins and Davies, 2019). This can result in changes to protein structure, function, and turnover and loss or occasional gain of activity (Hawkins and Davies, 2019). Moreover, oxidative stress can degrade lipids and carbohydrates into highly reactive intermediates, which eventually attack proteins at various functional sites (Kehm et al., 2021). The levels of aldehydic and ketonic derivatives

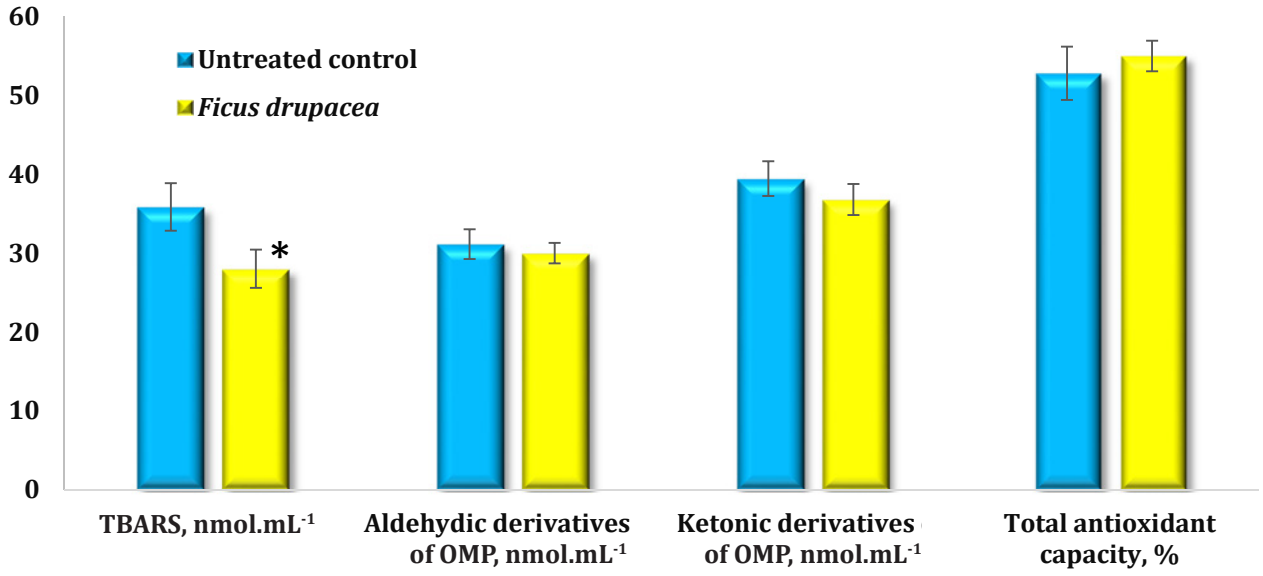

Figure 2 The TBARS content as a biomarker of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity in the equine erythrocytes after *in vitro* incubation with extract derived from leaves of *Ficus drupacea* Thunb. (M ±m, n = 18)
*– statistically significant differences between treated and untreated samples ($p < 0.05$)

of oxidatively modified proteins were also decreased in samples treated with an extract derived from leaves of *F. drupacea* compared to the untreated samples, but these decreases were statistically non-significant ($p > 0.05$). When equine erythrocytes were incubated with the extract derived from leaves of *F. drupacea*, the levels of aldehydic and ketonic derivatives were non-significantly decreased by 3.7% and 6.7% ($p > 0.05$). Also, a non-significantly increased TAC level was observed after incubation with an extract derived from leaves of *F. drupacea* (by 4.2%, $p > 0.05$) (Figure 2).

The activity of catalase, glutathione peroxidase, and ceruloplasmin level in the equine plasma after *in vitro* incubation with extract derived from leaves of *F. drupacea* was resented in Figure 3.

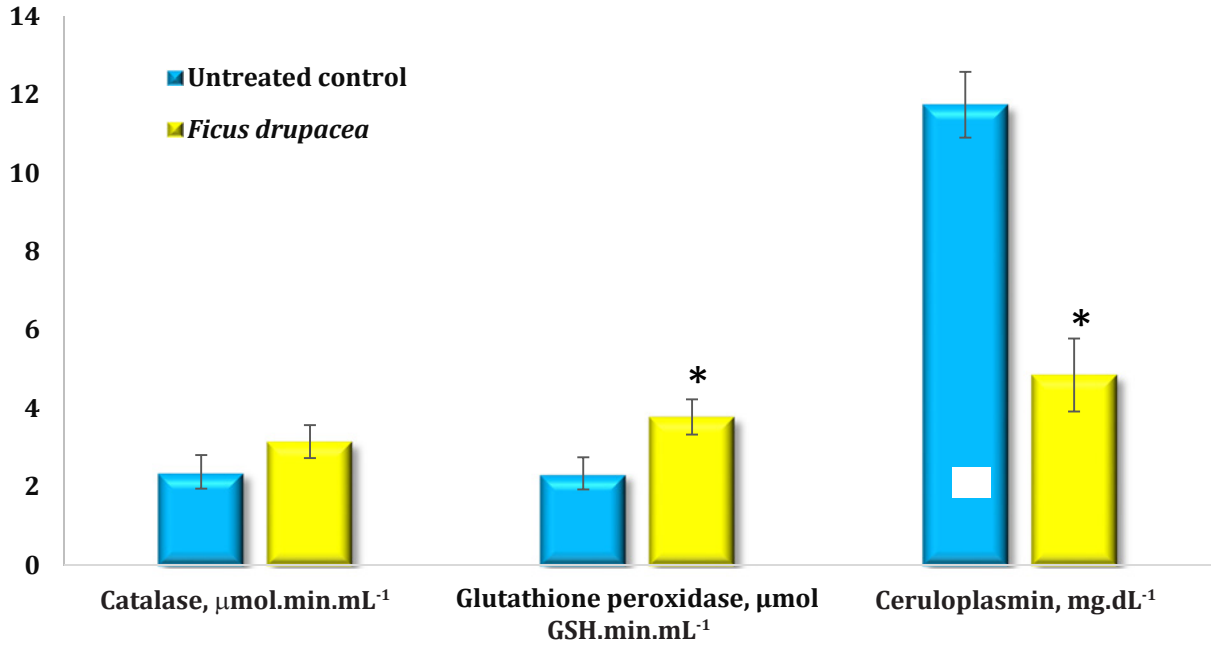

Figure 3 Activity of catalase, glutathione peroxidase, and ceruloplasmin level in the equine plasma after *in vitro* incubation with extract derived from leaves of *Ficus drupacea* Thunb. (M ±m, n = 18)
*– statistically significant differences between treated and untreated samples ($p < 0.05$)

Numerous short-lived and highly reactive oxygen species (ROS) such as superoxide ($O_{2\bullet}^{-}$), hydroxyl radical ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2) are continuously generated *in vivo* (Miao and St Clair, 2009). Superoxide dismutases (SODs), including copper-zinc superoxide dismutase (Cu, Zn-SOD), manganese superoxide dismutase(Mn-SOD), and extracellular (Ec-SOD) superoxide dismutase, play a crucial role in scavenging $O_{2\bullet}^{-}$ (Miao and St Clair, 2009). In the current study, SOD activity was increased to (430.39 ±34.15 $U.mL^{-1}$) in the equine plasma after *in vitro* incubation with an extract derived from leaves of *F. drupacea* compared to the untreated samples (303.96 ±29.51 $U.mL^{-1}$). This was a 41.6% (p = 0.000) increase in SOD activity compared to the untreated samples.

Catalases are well-studied enzymes that play critical roles in protecting cells against the toxic effects of hydrogen peroxide (Goyal and Basa, 2010). Catalase activity was no-significantly increased to value (3.15 ±0.42 μmol H_2O_2.min $.mL^{-1}$) in the equine plasma after *in vitro* incubation with an extract derived from leaves of *F. drupacea* compared to the untreated samples (2.38 ±0.43 μmol H_2O_2.$min.mL^{-1}$). This was a 32.4% (p >0.05) increase in CAT activity compared to the untreated samples (Figure 3).

Glutathione peroxidases (EC 1.11.1.9 and EC 1.11.1.12) catalyze the reduction of H_2O_2 or organic hydroperoxides to water or corresponding alcohols using reduced glutathione. Some glutathione peroxidase isozymes have a selenium-dependent glutathione peroxidase activity and present selenocysteine (Margis et al., 2008). Similarly to SOD and CAT activity, GPx activity was also increased to (3.78 ±0.45 μmol GSH.$min.mL^{-1}$) in the equine plasma after *in vitro* incubation with an extract derived from leaves of *F. drupacea* compared to the untreated samples (2.34 ±0.41 μmol GSH.min $.mL^{-1}$). This was a 61.5% (p = 0.000) increase in GPx activity compared to the untreated samples.

Ceruloplasmin (CP) is a serum ferroxidase that contains greater than 95% of the copper found in plasma. CP is a member of the multicopper oxidase family, an evolutionarily conserved group of proteins that utilize copper to couple substrate oxidation with the four-electron reduction of oxygen to water (Hellman and Gitlin, 2002). It has been proposed to function in copper transport, oxidation of organic amines, iron(II) oxidation, and the regulation of cellular iron levels, catechols, radical scavenging, and other antioxidant processes (Healy and Tipton, 2007). In the current study, CP level was decreased to (4.85 ±0.93 $mg.dL^{-1}$) in the equine plasma after *in vitro* incubation with an extract derived from leaves of *F. drupacea* compared to the untreated samples (11.74 ±0.84 $mg.dL^{-1}$). This was a 58.7% (p = 0.000) decrease in CP level compared to the untreated samples.

In the present study, we used an *in vitro* model of equine plasma and erythrocytes to assess the antioxidant properties of an aqueous extract derived from the leaves of *F. drupacea* leaves. Many results also clearly suggest that treatment by herbal extracts *in vivo* and *in vitro* studies prevents organ damage through a decrease of lipid peroxidation and protection of the antioxidant defence system. On this basis, the current study was conducted to evaluate the antioxidant properties of an extract derived from the leaves of *F. drupacea*. The main finding of the current study was that this extract was able to decrease both lipid peroxidation and protein damage, with a simultaneous increase in the activity of antioxidant enzymes (SOD, CAT, and GPx) in the equine erythrocytes and plasma after *in vitro* incubation.

The efficacy of *F. drupacea* on other *in vitro* and *in vivo* models was also investigated by other researchers. The efficacy of *Ficus* spp. on renal injury induced by hypercholesterolaemia was revealed by Awad et al. (2012a). The ethanol and hexane extracts of *F. microcarpa* L.f., *F. religiosa* L. and *F. mysorensis* B.Heyne ex Roth leaves were evaluated against renal injury induced by hypercholesterolaemia. For the *in vivo* study, all rats were orally given cholesterol (30 $mg.kg^{-1}$ body weight, BW) and leaf extract (500 $mg.kg^{-1}$ BW) five times per week for 9 weeks. Hypercholesterolaemic rats showed significant increases in urea nitrogen and creatinine while serum protein and albumin levels, nitric oxide (NO), Na^+, K^+-ATPase and phospholipids in kidney tissue were all decreased. Treatment with leaves extracts improved kidney function indices (urea nitrogen, creatinine, serum protein and albumin), kidney disorder biochemical parameters (NO, Na^+, K^+-ATPase and phospholipids), haematological profile (haemoglobin, RBCs and WBCs) and kidney histopathology. These researchers demonstrated that using *Ficus* spp. resulted in improving renal injury induced by hypercholesterolaemia, with the most potent effects seen while using *F. microcarpa* hexane extract (Awad et al., 2012a). Also, these researchers (Awad et al., 2012b) screened some *Ficus* and *Morus* spp. for hypolipidaemic and antioxidant activities and *in vivo* assessment of *F. mysorensis* and revealed that *F. mysorensis* demonstrated hypolipidaemic and antioxidant effects.

In an *in vitro* study, the ethanolic and hexane extracts of the investigated plants were evaluated against

hyperlipidaemia by estimating the rate-limiting enzyme of cholesterol biosynthesis; β-hydroxy-β-methylglutaryl coenzyme A reductase (HMG-CoA reductase). The antioxidant activity was evaluated by the reduction of DPPH(-) free radicals. Extra phytochemical screening of *Ficus* extracts was undertaken, which recorded potent hypolipidaemic and antioxidant activities. The more pronounced extract, *F. mysorensis* (hexane extract), was evaluated *in vivo* by estimation of the lipid profile and certain antioxidant parameters in hypercholesterolemic rats. The hexane fraction was chromatographed and six isolated compounds were identified (Awad et al., 2012b).

Yessoufou et al. (2015) investigated the antimicrobial (fungi and bacteria) and antiproliferative activities of crude extracts of the *F. drupacea* stem bark and isolated compounds from *F. drupacea*. Stem bark extracts of *F. drupacea* and the isolated compounds represent potential antibacterial and antifungal resources against a wide spectrum of microbes. Seven biochemical compounds from stem bark extracts including β-amyrin (1), β-sitosterol-3-O-β-D-glucopyranoside (2), 5-O-methyllatifolin (3), oleanolic acid (4), epifriedelanol (5), friedelin (6) and epilupeol acetate (7) were isolated and identified. Of all the seven compounds, compounds 3 and 7 exhibited the highest antifungal and antibacterial activities against screened microorganisms (Yessoufou et al., 2015).

Thus, in the current study, we have undertaken an attempt to investigate the *in vitro* antioxidant activity of an extract derived from the leaves of *F. drupacea* plants. The results obtained suggested that antioxidant compounds are dominant contributors to the antioxidant activity of the extract derived from the leaves of *F. drupacea* plants. Our future phytochemical screening of leaves also will reveal the presence of various classes of secondary metabolites which have great importance in medicinal chemistry and natural product research for their high antioxidant properties.

Conclusions

In the current study, we investigated the changes in the oxidative stress biomarkers and antioxidant defences using the model of equine erythrocytes and plasma to evaluate the antioxidant activities of the aqueous extract derived from the leaves of *F. drupacea*. The treatment of equine erythrocytes by extract derived from leaves of *F. drupacea* resulted in reduced lipid peroxidation and oxidatively modified protein. The levels of aldehydic and ketonic derivatives of oxidatively modified proteins were non-significantly decreased. The incubation of equine plasma with an extract derived from leaves of *F. drupacea* increased antioxidant defences. The level of total antioxidant capacity was non-significantly increased. However, further detailed investigation, especially *in vivo* and *in vitro* antioxidant studies is needed to justify the use of extract derived from leaves of *F. drupacea* as a natural source of antioxidants.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

Funding

This work was supported by The International Visegrad Fund, and the authors are cordially grateful for this.

Acknowledgement

This research was supported by the Pomeranian University in Słupsk (Poland).

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